# The population structure of roundnose grenadier (*Coryphaenoides rupestris*) in southwestern Norway

**Thesis submitted in partial fulfilment of the requirements for the degree**

**Master of Science in Marine Biology**

**By**

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Cover image: a misty autumn morning during fieldwork in Masfjord aboard the RV Håkon Mosby.

# Abstract

Ocean circulation, bathymetric barriers, and ecological processes can hinder the dispersal of marine fishes and thus generate sub-populations. The present study investigated the population structure of a benthopelagic fish, *Coryphaenoides rupestris*, from three Norwegian fjords and two coastal sites using eight microsatellite DNA markers. Genetic analyses revealed significant population genetic structure across the study area ($F_{ST}$ = 0.0297, P < 0.001) and temporal stability in the Skagerrak. There was evidence of highly isolated sub-populations, as shown by significant pairwise differences in tests of genic differentiation and analysis of molecular variance (AMOVA), high inbreeding coefficients ($F_{IS}$), high homozygosity, and low genetic diversity. Small-scale, within-fjord population structuring was also found in Lustrafjord. Mantel tests revealed a strong effect of isolation by distance and isolation by depth (bottom depth) and a possible effect of bottom temperature. Significant differences in fish condition were found between sites and included length-weight relationships (Analysis of Covariance (ANCOVA): F = 8.249, df = 7, P < 0.001), Hepatosomatic Index (HSI; GLM: F = 252.48, df = 3, P < 0.001) and Gonadosomatic Index (GSI; GLM: F = 15.91, df = 3, P < 0.001). In conclusion, population structuring in *C. rupestris* along the Norwegian coast seems to be influenced by distance, bathymetric barriers like bottom depth and fjord sills, and differences in fish condition indicate possible differences in environmental conditions between sites. *Coryphaenoides rupestris* is an overfished species that has been red-listed as critically endangered. Based on the present findings, stock management should consider each of the sub-populations independently, and not depend on recovery through recruitment from neighbouring sub-populations.

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# Introduction

Connectivity among marine fish populations is generally thought to be high because there are relatively few barriers to dispersal and migration in the marine environment (Ward et al. 1994). While population structure signals may generally be weak in marine species, they still exist to varying degrees depending on a species' biology and environment. While fishes with long pelagic egg and larval stages are generally thought to disperse widely with ocean currents (Knutsen et al. 2007a), small-scale population structuring is known to occur in many of these species. Isolated sub-populations can occur within pockets along Norway's complex coast, within archipelagos and fjords, in fishes such as cod (Jorde et al. 2007; Knutsen et al. 2007b), mesopelagic fishes (Kristoffersen and Salvanes 2009; Suneetha and Nævdal 2001), and herring (Aasen 1952). The complexity of Norway's coastal seas provides a unique environment to study population structuring in a benthopelagic fish that has pelagic early life stages and is commonly found in deep fjords.

Studies of population structure have frequently contributed to the stock management of commercially important fishes like cod (Wennevik et al. 2008; André et al. 2016) and Atlantic herring (Mariani et al. 2005). Declining shallow fish resources, increasing demand for these resources and improved fishing technologies have led to the deepening of fishing grounds and the commercial harvest of deep-sea fishes since the 1950s (Morato et al. 2006). Many deep-sea fishes have life-history characteristics that are not conducive to intensive exploitation, such as slow growth and late age at maturity, and have already experienced population declines (Devine et al. 2006; Devine et al. 2012). Research on the biology and population structure of commercially important deep-sea fishes is therefore required, but has only recently come to the fore in species like orange roughy (Varela et al. 2013), redfish (Valentin et al. 2014), and roundnose grenadier (Knutsen et al. 2012).

The roundnose grenadier (*Coryphaenoides rupestris*) is a benthopelagic fish occurring across the North Atlantic and along Norway's southwestern coast and fjords (Figure 1). Commercial fishing of roundnose grenadier, coupled with its slow life-history characteristics, has led to its depletion and subsequent listing as critically endangered on IUCN's Red List (Iwamoto 2015). This has led to the establishment of strict management practices, including a moratorium in the Skagerrak since 2006 (Appendix 1). Two general theories exist for its reproductive pattern. The first is that
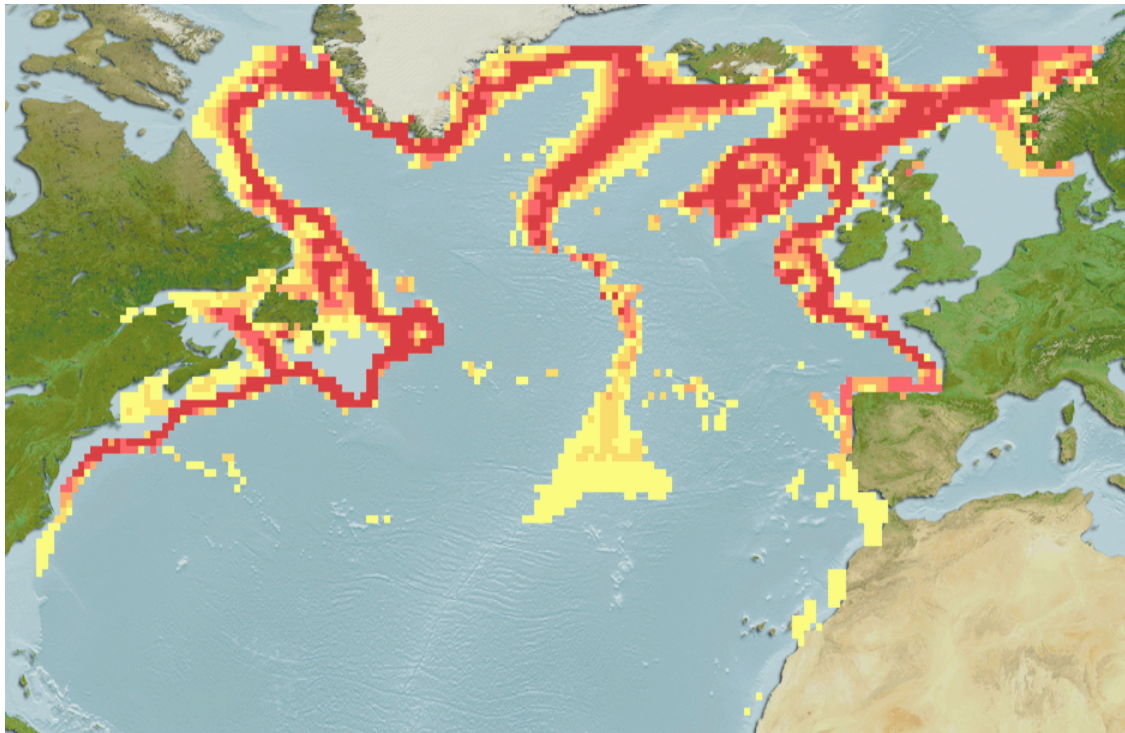
Figure 1: The global distribution of *Coryphaenoides rupestris*, taken from www.fishbase.org.

spawning occurs in Icelandic waters, after which eggs and larvae are distributed across the Atlantic by ocean currents and return to their spawning grounds as adults. The second theory is that spawning stocks are separate at the Mid-Atlantic Ridge (MAR), the northeast and northwest Atlantic (Atkinson 1989; Atkinson et al. 1982, and references therein). Studies on roundnose grenadier have supported the latter theory and indicated even finer scale population structuring occurring along the Canadian coast, the MAR, and to the west of the British Isles, as demonstrated using length-weight analyses (Bergstad 1990; Atkinson 1989; Vinnichenko and Khlivnoi 2007; O'Hea et al. 2013), otolith microchemistry (Longmore et al. 2010, 2011), and genetic studies (Logvinenko et al. 1983; White et al. 2010; Knutsen et al. 2012). In Norwegian waters, evidence exists of isolated sub-populations in the Skagerrak (Bergstad 1990; Longmore et al. 2010; Longmore et al. 2011; Knutsen et al. 2012) and Trondheimsleia (Knutsen et al. 2012), both of which are deep basins surrounded by shallower sills.

Roundnose grenadiers live for up to 72 years (Devine et al. 2012) but it is unknown whether this allows for dispersal over time. They are thought to be weak swimmers and dispersal is most likely to occur during their lengthy (up to 6-7 months) pelagic egg, larval and juvenile stages (Knutsen et al. 2012; Bergstad and

Gordon 1994). They are also known to move up and down the water column to feed (Bergstad and Gordon 1994; Bergstad 1990; Haedrich 1974), which would put them into contact with different currents and aid in dispersal. The considerable time spent high in the water column during early life stages may allow for long-range movement via ocean currents. However, dispersal in Norwegian waters has been hypothesised to be limited by shallow bottom features such as sills (Bergstad 1990).

Fjords are long, narrow, deep inlets carved out by glaciers and filled with seawater after the last glacial maximum around 17,000 years ago. They often have a shallow sill at the entrance that limits the circulation of deep basin waters, while waters above sill depth flow freely in and out of the fjords (Syvitsky et al. 1987). A large volume of water is exchanged between fjords and the Norwegian Coastal Current (NCC, Figure 2A), driven by coastal winds and the characteristics of each fjord. These processes enable the transport of plankton in and out of fjords (Asplin et al. 1999). If pelagic early-life stages of fishes are present in the upper advective layers of fjords (above sill depth), they could disperse into, out of, and between fjords depending on wind and ocean circulation patterns.

The present study will determine the connectivity of roundnose grenadier among southwestern Norwegian fjords. Based on population structuring patterns found in other fishes along Norway's coast and on roundnose grenadier's benthopelagic habits, we predict that population structure will be found along Norway's coast because of bathymetric dispersal barriers. We believe this information will provide valuable information for future stock assessments to ensure the sustainable management of the species.

## Aims and hypotheses

The aim of this Master's project is to determine the population structure of *Coryphaenoides rupestris* in southwestern Norway, focusing on fjord and coastal sites. Microsatellite DNA markers will be used to test for population genetic structure across space and time, testing the null hypothesis ($H_0$): there are no spatial or temporal differences between or among sites, such that all sampled individuals are part of a single, panmictic population unit. Under the null hypothesis, there are no significant differences in allele frequencies or differences in heterozygote frequencies (such that the fixation index $F_{ST} = 0$) between sites and years. I will also investigate

the potential causes of population differentiation using Mantel correlation tests. To do this, I will test whether measures of genetic distance between sites are correlated with abiotic (geographical distance, bottom depth, sill depth, salinity, oxygen, temperature) and biotic (morphological and condition indices) variables.

Finally, I will test for differences in fish condition between sites using three indices: a morphological index using length-weight regressions, a comparison of energy reserves using the Hepatosomatic Index (HSI), and a comparison of reproductive potential using the Gonadosomatic Index (GSI). HSI and GSI will only compare fjord sites because no HSI or GSI data was collected in Trondheimsleia or the Skagerrak. These indicators will test the null hypothesis ($H_0$): there is no difference in the condition of grenadiers between sites. The results will indicate whether there may be differences in the living environment of grenadiers between sites.

## Materials and Methods

### Species

The roundnose grenadier (*Coryphaenoides rupestris*, Family Macrouridae, Order Gadiformes) is distributed across the continental, island, and seamount slopes of the north Atlantic and Mid-Atlantic Ridge (MAR, Figure 1). The species has been recorded between 180 m and 2200 m (FAO 2016; Knutsen et al. 2012). Roundnose grenadiers are long-lived (maximum 50-72 years), slow growing, and late maturing, with 50% of individuals reaching maturity between 8 and 14 years old (Devine et al. 2012). These life-history parameters vary across their distribution range (Bergstad 1990; Bergstad and Gordon 1994; Allain 2001). Eggs are probably spawned near the sea floor and then move up the water column; eggs have been observed between 150 m and 600 m deep (Merrett 1978; Lorance et al. 2008). Their pelagic phase continues into the larval and early juvenile stages, during which time they move to deeper and colder waters with increasing size and eventually adopt a benthopelagic lifestyle (Longmore et al. 2011; Merrett 1978). In the Skagerrak, this takes 6 or 7 months, but is thought to vary across its distribution range (Knutsen et al. 2012; Bergstad and Gordon 1994).

## Sampling sites

*Coryphaenoides rupestris* have been collected using bottom and pelagic trawls from Trondheimsleia, Lustrafjord, Masfjord, Korsfjord and the Skagerrak (Table 1, Figure 2). The sites include channels, fjords, and more open inlets (extensions) of the North Sea. Additional sampling in the Norwegian Trench was attempted with the RV G.O. Sars on 25 November 2015, but these failed to capture any specimens.

Trondheimsleia is a channel located between the Norwegian mainland and the islands of Hitra and Smøla, just west of Trondheim. Like the other sites, it is a deep basin surrounded by shallow sills (Figure 2B). The Institute of Marine Research (IMR) in Flødevigen has provided samples from Trondheimsleia from 2004.

Lustrafjord is a northward fjord arm located deep within Sognefjord, Norway's longest (200 km) and deepest (1308 m) fjord. Lustrafjord consists of several basins separated by sills, progressively deepening into Sognefjord like a giant staircase (Syvitsky et al. 1987; Aasen 1952). To test for small-scale population structuring and structuring by depth in a localised area, grenadiers were collected from an inner fjord basin (375 m) and an outer fjord basin (650 m, Figure 2C), which

Table 1: Characteristics of the sampling sites, including bathymetry and CTD (temperature, salinity, oxygen) data at the time of sampling. Coordinates shown for one of the sampling stations at each site. Coordinates for all genetic samples are given in Table 3. Site name abbreviations are shown for reference in later tables in graphs.

| Site | Trondheimsleia | Lustrafjord (Inner) | Lustrafjord (Outer) | Masfjord | Korsfjord | Skagerrak |
|---|---|---|---|---|---|---|
| **Abbreviation** | TL | Lus-In | Lus-Out | Mas | Kor | 2001: SK01 2008: SK08 2016: SK16 |
| **Latitude (decimal)** | 63.43360 | 61.36028 | 61.23336 | 60.88447 | 60.18981 | 58.22600 |
| **Longitude (decimal)** | 8.66080 | 7.38415 | 7.37407 | 5.45101 | 5.23125 | 9.54600 |
| **Bottom depth (m)** | 270 | 375 | 650 | 494 | 690 | 700 |
| **Sill depth (m)** | 100 | 320* | 570** | 75 | 250 | 275 |
| **Length (km)** | 400 | 40 | 40 | 20 | 15 | 240 |
| **Bottom temperature (°C)** | N/A[#] | 7.6 | 7.4 | 2015: 8.2 | N/A | 2001: N/A[#] 2008: 6.5 2016: 6.6 |
| **Bottom salinity (PSU)** | N/A[#] | 35.0 | 35.0 | 2015: 35.1 | N/A | 2001: N/A[#] 2008: 35.2 2016: 35.2 |
| **Bottom O2 (mg L-1)** | N/A[#] | 3.5 | 3.7 | 2015: 2.4 | N/A | 2001: N/A[#] 2008: 5.0 2016: 2.9 |

[#] CTD data unavailable.
\* Depth of sill between Inner and Outer Lustrafjord basins.
\*\* Depth at the entrance of Lustrafjord from Sognefjord.
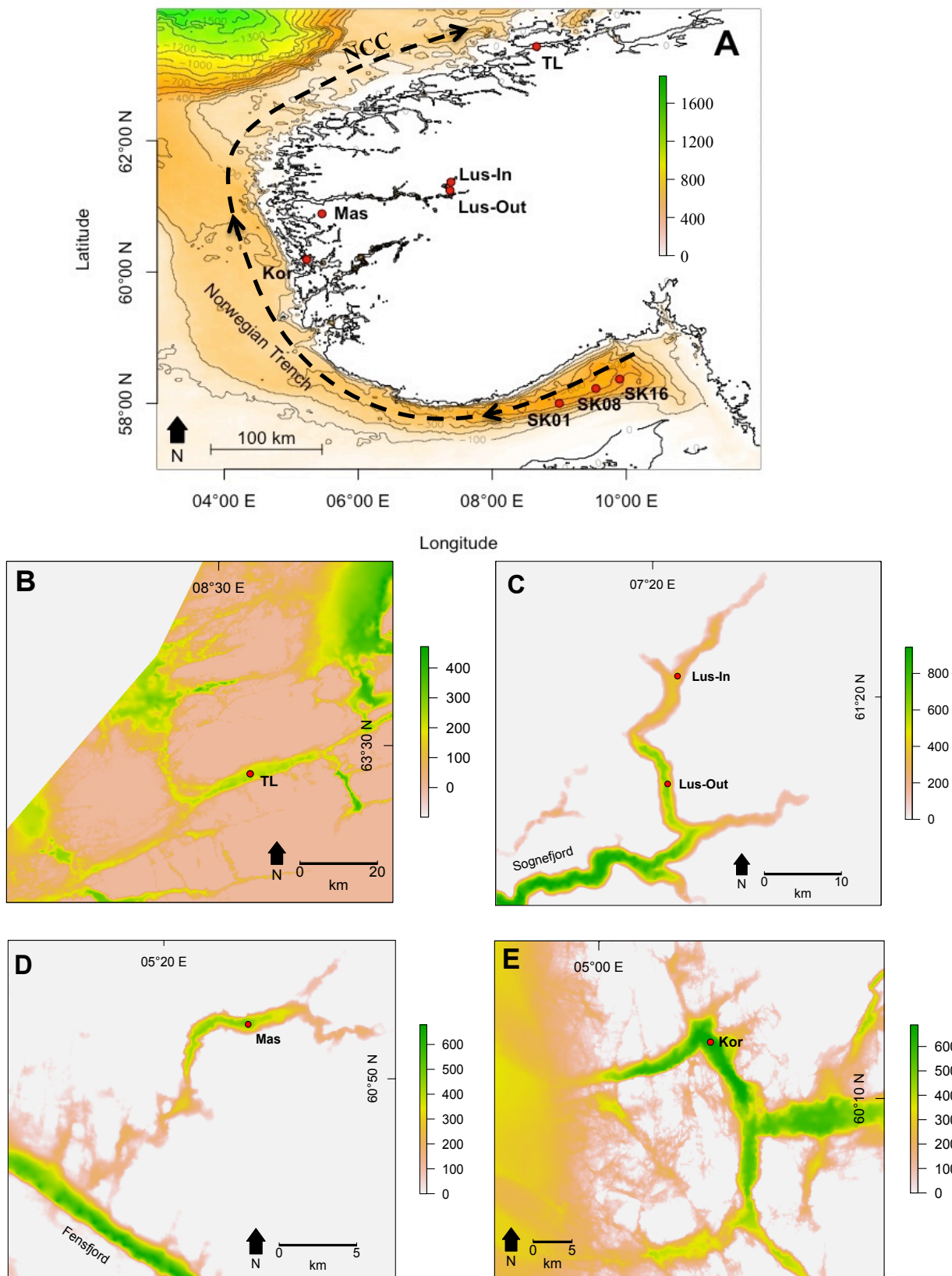
Figure 2: A) Bathymetric map of southwestern Norway showing the sampling stations for *Coryphaenoides rupestris*. The dotted arrow shows the direction of the Norwegian Coastal Current (NCC). Magnified sites are B) Trondheimsleia C) Inner and Outer Lustrafjord D) Masfjord and E) Korsfjord. Colour scale represents bottom depth in metres. Map A was produced using marmap (Pante and Simon-Bouhet 2013), and maps B-E using data provided by IMR. The grey area in map B indicates the limit of the bathymetric survey.

are separated by a 320 m sill. Samples were collected during two cruises to Lustrafjord aboard the RV Håkon Mosby (trawl opening ~60 m) in July and September 2016.

Masfjord, located approximately 50 km north of Bergen, is a fjord arm separated from the larger Fensfjord by a 75 m deep sill (Figure 2D). The RVs Håkon Mosby and G.O. Sars have trawled in Masfjord each autumn from 2011 to 2015 as part of an on-going field course by the University of Bergen. Samples from 2012 and 2015 were used for condition indices and samples from 2015 were used for genetic work.

Korsfjord is located south-west of Bergen. Korsfjord has an approximately 250 m deep sill towards the Norwegian Trench to the west and connects to two inner fjords to the north and south with sills at 150 m and 450 m (Figure 2E). Seasonal flushing occurs with oceanic waters from the Norwegian Trench, allowing Korsfjord's deep basin water to retain oceanic characteristics like high oxygen and salinity (Bakke and Sands 1977; Matthews and Sands 1973). Grenadiers were collected in Korsfjord aboard the RV Hans Brattström (trawl opening ~12 m) in August 2016.

The Skagerrak, a channel between the North Sea and Kattegat, is a semi-isolated deep basin with a cyclonic circulation pattern (Bergstad and Gordon 1994). IMR has performed annual shrimp surveys in the Skagerrak since 1984, in which *C. rupestris* have been caught as bycatch, and have provided samples from 2001, 2008 and 2016 (Figure 2A).

The opportunistic nature of sampling across all study sites meant it was not possible to obtain fully random samples. Thus, the specimens collected may not be representative of the whole populations. However, this was the best that could be obtained given the difficulty of sampling and scarcity of sampling opportunities. The sample sizes collected (Table 2) should allow sufficient statistical power to fulfil the aims of the study (Ryman et al. 2006).

Station data, consisting of 40 variables, were collected following IMR protocol and included date, time, position, depth, and vessel. CTD data (salinity, temperature, oxygen) were also taken. However, due to gear and time constraints only position, depth, date, and time were taken when sampling in Korsfjord. No CTD data were available for Trondheimsleia and Skagerrak in 2001.

## Sampling procedure

The sampling procedure was aimed at gathering genetic material and biological data, and was similar at all sites. Grenadiers were sorted from the bottom trawl catch (Figure 3A-C), rinsed if they were covered in biological material or sediment from the trawl, and either processed immediately on board or frozen for later processing in the laboratory.

Tissue for genetic analysis was taken from at least 100 individuals at each site, except for Lustrafjord where these were divided between the inner and outer fjord (Table 2). Genetic samples were mainly collected as a dorsal fin clip. Tail clips were collected where fin clip tissue was limited (e.g. juveniles and individuals with damaged dorsal fins from trawling). Muscle tissue was provided by IMR for Trondheimsleia and Skagerrak samples. Scissors and forceps were wiped with ethanol and tissue paper in between samples to avoid cross-contamination of genetic material between individuals. All samples were preserved in Eppendorf tubes in 99+ % ethanol and kept in a -18°C freezer upon delivery to the Department of Biology.

Additional data sampled from the grenadiers differed among sites (Table 2). Masfjord and Korsfjord grenadiers were frozen and processed at the University of Bergen's Department of Biology laboratory, and those from Lustrafjord assessed on

Table 2: Overview of the raw sampling material, with sample sizes shown (N) shown as "Year: N".

| Locality | Trondheimsleia | Lustrafjord (Inner) | Lustrafjord (Outer) | Masfjord | Korsfjord | Skagerrak |
|---|---|---|---|---|---|---|
| **Genetics** | 2004: 96 | 2016: 48 | 2016: 48 | 2015: 96 | 2016: 96 | 2001: 40 2008: 39 2016: 17 |
| **Length-weight analysis** | 2004: 100 | 2016: 48 | 2016: 179 | 2012: 75* 2015: 131* | 2016: 108 | 2001: 299 2008: 100 2016: 17 |
| **Sex[#]** | 2004: 100 | 2016: 30 | 2016: 126 | 2012: 16 2015: 37 | 2016: 76 | 2001: 298 2008: 0 2016: 17 |
| **Sexual maturity** | 2004: 100 | 2016: 47 | 2016: 158 | 2012: 8 2015: 45 | 2016: 95 | 2001: 299 2008: 0 2016: 17 |
| **Liver weight** | N/A | 2016: 48 | 2016: 158 | 2012: 75 2015: 130 | 2016: 107 | N/A |
| **Gonad weight** | N/A | 2016: 31 | 2016: 122 | 2012: 71 2015: 113 | 2016: 86 | N/A |
| **Gutted weight** | N/A | 2016: 29 | 2016: 151 | 2012: 75 2015: 131 | 2016: 108 | N/A |
| **Otoliths** | N/A | 2016: 48 | 2016: 179 | 2012: 75 2015: 131 | 2016: 108 | N/A |

* Grenadiers from the BIO310 research cruises that have been re-measured and processed.
[#] Sexual maturity could be determined in some cases where sex was unknown (e.g. immature juveniles).

Figure 3: An overview of the sampling method for *Coryphaenoides rupestris*. A) A bottom trawl is hauled aboard the RV Håkon Mosby in Lustrafjord. B) One of many grenadiers from Lustrafjord with long first pelvic fin rays. C) Grenadiers sorted from a catch prior to rinsing and processing. D) Measuring the pre-anal fin length of a grenadier. E) Dissection of a mature female showing the liver, stomach, and gonads full of eggs. D) Resting stage male gonads.

board the research vessel. Total weight (0.01 g precision), pre-anal fin length (PAFL,

0.5 cm precision, Figure 3D), liver and gonad weight (0.1 g precision, Figure 3E), and

sex and maturity, assessed according to IMR protocol (Mjanger et al. 2016, Figure

3E-F) were measured for all individuals. The assessment of sex and maturity for many grenadiers was uncertain, in which case, these were labelled as 'NA'. Otoliths were also collected for future graduate studies. Stomach content, sex and maturity data were not used in this study. Stomachs, livers and gonads from a subset of Masfjord and Lustrafjord samples were frozen for use in other research. For Trondheimsleia and Skagerrak grenadiers, PAFLs (rounded down to 1 cm precision) and weights (rounded down to 1 g precision) were provided, as well as sex and maturity for some samples, by IMR staff in Flødevigen.

Many of the grenadiers collected in Lustrafjord had a very long outer pelvic fin ray, up to two or three times the head length (Figure 3B). This raised questions as to whether the Lustrafjord populations of grenadier might be unique. A few individuals sampled at Korsfjord at a later date showed the same trait. It is unknown whether this trait is present across other populations because we were not actively looking for it; therefore, we may have missed recording it at other sites. In addition, fish are damaged in the trawl, particularly fragile structures such as fin rays and tails. That some specimens had a longer outer pelvic fin ray may be because these individuals sustained less damage in the trawl.

## Genetic analysis procedure

DNA extractions, polymerase chain reactions (PCR), and dilutions were performed at the Marine Biodiversity Laboratory at the Department of Biology, University of Bergen. Sequencing and scoring of alleles was performed at the IMR's DNA-laboratory in Bergen. Ninety-six individuals from each locality were used for microsatellite analysis. Those from Lustrafjord were divided between the inner and outer fjord, and those from Skagerrak were divided between years (Table 2). These numbers were selected for practical and cost-effective reasons and optimised to the arrangement of sequencing plates containing 96 wells.

Nineteen microsatellite primers have been developed for *C. rupestris*, and are listed in Appendix 2 (Knutsen et al. 2008; Schneider et al. 2009; White et al. 2009). Ten primers were ordered from Applied Biosystems UK, dried at 80 nmol with their forward sequences marked with NED, VIC, FAM or PET fluorescent tags (Appendix 2). These were stored in a -18°C freezer upon delivery to the laboratory. Stock

solution was prepared by diluting the primers to 100 μmol in TE buffer and stored in a -18°C freezer.

## Genetic pilot study

A pilot study testing the genetic laboratory procedure was performed on 16 individuals (Sk01-Sk16) from the 2016 Skagerrak samples. First, the presence of DNA after extraction using the hot sodium hydroxide and tris (HotSHOT, see details in DNA extraction section below) method was tested. A standard 100 mL electrophoresis gel was run and DNA bands were clear for all individuals. The presence of DNA was also detected in a NanoDrop ND-1000 Spectrophotometer. Next, polymerase chain reactions (PCRs) and sequencing were performed (see below sections for method) to test each primer on these 16 individuals. Adjustments were made through trial and error, by adjusting the amount of tissue to use for DNA extraction, adjusting the concentrations of primer user solutions and DNA extracts to use for PCR, multiplexing (combining in PCR) primers in different combinations, and running PCRs at different annealing temperatures and number of cycles. After a few rounds of trial and error, the alleles at most loci could be seen and scored consistently in the sequencing output, so the full-scale runs were started. To save on costs, the protocol was further optimised during the first two full-scale runs. Despite several adjustments to the procedure, the loci Crup8 and CorRu7 maintained a low success rate (<30 % of alleles could be scored), with weak readings and high background noise in the sequencing output (Figure 4E-F). These were eliminated from the study, so that eight loci were used in the end. The final optimised protocol is described below.

## DNA extraction

DNA was extracted from a small (a few millimetres) piece of tissue. The tissue was cut on pieces of printing paper to drain ethanol from the tissue. Between each sample, the paper was replaced and the scalpel and forceps were wiped with ethanol. DNA isolation was performed using hot sodium hydroxide and tris (HotSHOT), a method developed by Truett et al. (2000). The samples were heated to 95 °C for 30 minutes and then cooled to 4 °C in a MJ Research PTC-200 Peltier Thermal Cycler (Figure 4A). DNA extracts were stored in a 4 °C refrigerator.

**Pre-PCR**

User solutions of 10 μmol were prepared by diluting stock solution in TE buffer. Five master mixes were prepared using a standard TaKaRa Ex HS kit (Appendix 3). For the PCRs, 1 μL of DNA extract was added to 24 μL of master mix. Master mix 1 was a 'true' multiplex (multi-1), while master mixes 2 to 5 were combined post-PCR to form a 'false' multiplex (multi-2).

**Polymerase Chain Reaction (PCR)**

Five PCRs were performed per individual in Bio-Rad S1000$^{TM}$ and C1000$^{TM}$ Thermal Cyclers (Figure 4B). The same basic setting was used for each PCR; the differences lay in the annealing temperatures and number of cycles (Appendix 4). A 30-minute period of 60 °C at the end of the protocol was added to strengthen the A-plus peaks, which are peaks detected at one base pair higher than the allele, to facilitate allele scoring. The PCR products were diluted prior to sequencing (Figure 4C). Multi-1 was diluted in double-distilled $H_2O$ at a 1:15 ratio. Multi-2 combined 2 μL from the products of PCRs 2 through 5. The diluted PCR products were stored at 4 °C in darkness for no longer than two days, or frozen at -18 °C for no longer than three days, before sequencing.

**Sequencing and allele scoring**

A mixture comprising of 8 μL volume of GeneScan$^{TM}$ 500 LIZ$^{TM}$ size standard and 800 μL of Hi-Di$^{TM}$ Formamide was prepared at the IMR. 8 μL of this mixture was loaded into sequencing plates with 2 μL of the diluted PCR products (10 μL volume per individual and PCR-product). The plates were analysed in an Applied Biosystems ABI 3730 DNA Analyzer. Binning and allele scoring was performed in GeneMapper Software 5 (Figure 4D-F). For samples that showed weak or no peaks, high background noise or were scored with uncertainty, PCR and sequencing were repeated. For individuals with low scoring success across all loci, the entire process was re-run using new DNA extracts. After gaining experience in allele scoring throughout the runs, early runs were re-assessed to ensure a consistent scoring method. For loci with consistently strong A-plus peaks, the A-plus peak was scored. The alleles' true peaks were scored for the remaining loci.
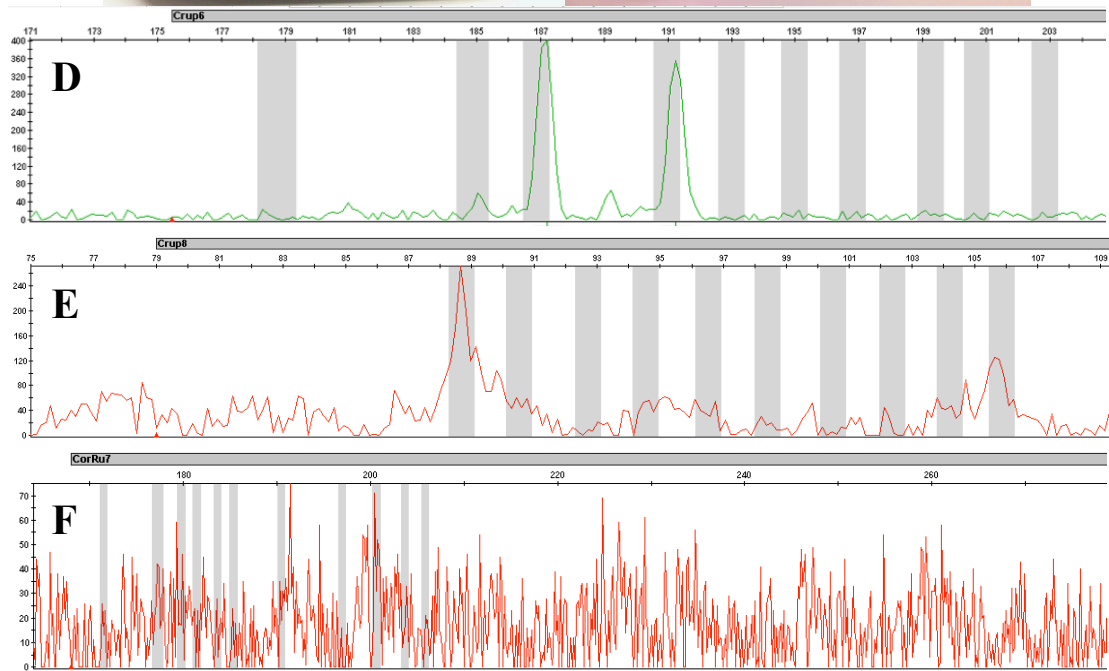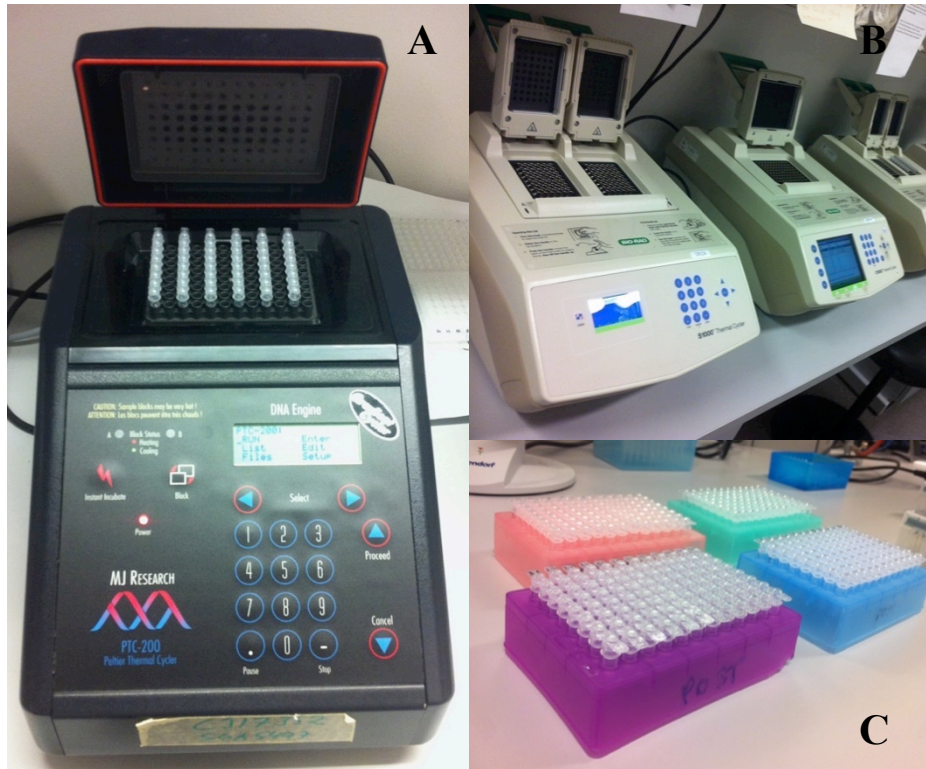
Figure 4: An overview of the genetic laboratory procedure. A) 48 individual tissue samples ready for extraction using HotSHOT in a MJ Research PTC-200 Peltier Thermal Cycler. B) Bio-Rad S1000[TM] and C1000[TM] Thermal Cyclers used for PCR. C) PCR products ready for dilution. D-F) Examples of 3 loci viewed in GeneMapper: a quality output of a heterozygote at the locus Crup6 (D), and outputs with weak peaks and high background noise at Crup8 (E) and CorRu7 (F) that could not be scored.

## Statistical Analysis

### Population genetic analysis

The scoring success of loci ranged from 80% to 95% (Appendix 5). Individuals with more than three non-scored loci were excluded from the analyses to minimise the number of empty cells in the data (resulting sample sizes in Table 3), which could skew the results of the following tests. Large sample sizes were maintained even with the exclusion of these individuals.

Table 3: Sample size for genetic analysis after the removal of individuals with more than three non-scored loci. Sampling sites, years, and decimal coordinates are shown.

| Site | Year | Latitude, Longitude (decimal degrees) | Sample size |
|---|---|---|---|
| Trondheimsleia | 2004 | 63.43360, 8.66080 | 93 |
| Inner Lustrafjord | 2016 | 61.36936, 7.38336 | 10 |
| | | 61.37393, 7.38869 | 3 |
| | | 61.37719, 7.39274 | 12 |
| | | 61.35988, 7.38362 | 5 |
| | | 61.36028, 7.38415 | 4 |
| | | 61.36476, 7.38447 | 6 |
| | | | Total = 40 |
| Outer Lustrafjord | 2016 | 61.24258, 7.36912 | 29 |
| | | 61.28398, 7.31799 | 4 |
| | | 61.25310, 7.36612 | 2 |
| | | 61.23171, 7.36873 | 2 |
| | | | Total = 37 |
| Masfjord | 2016 | N/A | 7 |
| | | 60.88756, 5.45828 | 13 |
| | | 60.88017, 5.43741 | 3 |
| | | 60.88154, 5.43607 | 1 |
| | | 60.87977, 5.43311 | 1 |
| | | 60.88418, 5.45550 | 43 |
| | | 60.88447, 5.45101 | 13 |
| | | | Total = 81 |
| Korsfjord | 2016 | 60.18981, 5.23125 | 95 |
| Skagerrak | 2001 | 58.00000, 9.00000 | 39 |
| | 2008 | 58.22600, 9.54600 | 38 |
| | 2016 | 58.37000, 9.90000 | 17 |
| | | | Total = 94 |

The number of alleles and observed and expected heterozygosities for each locus at each site were computed in GenAlEx (Genetic Analysis in Excel version 6.5, Peakall and Smouse 2012; Peakall and Smouse 2006). Allelic richness was calculated in FSTAT (Goudet 1995). Deviations from Hardy-Weinberg equilibrium were tested for each site at each locus by calculating their inbreeding coefficients ($F_{IS}$, Weir and

Cockerham 1984) and associated P-values using a Hardy-Weinberg exact probability test in GenePop, using GenePop's default settings (version 4.6, Rousset 2008). To control for type I errors (false positives) that may arise from multiple testing, the false discovery rate (FDR) method of Benjamini and Hochberg (1995) was applied. P-values were ordered and ranked from smallest to largest. Significant P-values were accepted when $P_i \leq \frac{i}{m} \times 0.05$, where $i$ is the rank and $m$ is the number of significance tests performed (64 with all eight loci, or 32 with four loci). Where significant deviations from Hardy-Weinberg were found, individuals that were homozygous for rare alleles (alleles with <5 % frequency) were identified. A new Hardy-Weinberg exact probability test was performed using FDR correction without these individuals to test whether Hardy-Weinberg had been influenced by these rare genotypes.

Micro-Checker (version 2.2.3, Van Oosterhout et al. 2004) was used to check for null alleles, large allele dropout, and stuttering. Potential null alleles resulting from an excess of homozygotes were found at six loci across several sampling sites. These were CorRu3 (7 sites), CorRu11 (7 sites), Crup1 (5 sites), CorRu2 (4 sites), CorRu33 (1 site), and CorRu12 (1 site). Possible stuttering was detected at CorRu3 for Trondheimsleia. The presence of candidate loci under positive or balancing selection was tested using LOSITAN-selection workbench (Beaumont and Nichols 1996; Antao et al. 2008) with the default parameters and fifty thousand simulations. The assessment is a function of the relationship between heterozygosity and the fixation index ($F_{ST}$, see below) at each locus across all individuals. One locus, CorRu3, was a candidate for positive selection. To test whether potentially problematic loci (potential null alleles for several sampling sites, and candidate loci under selection) may influence the results, tests for Hardy-Weinberg equilibrium, genic differentiation, and analysis of molecular variance (AMOVA) were performed twice: once with all eight loci, and once after removing Crup1, CorRu2, CorRu3 and CorRu11.

To test for population differentiation among all samples, a G-test was performed with the software Genepop (G-test and default settings). The test was also applied for all pairs of sites. To control for type I error rates in the pairwise tests, an adaptation of the FDR correction by Benjamini and Yekutieli (2001) was used ($m = 28$). This method is a simple correction to account for correlation among tests, where the number of significant P-values satisfies the equation $P_i \leq \frac{i}{m} \times \frac{0.05}{\sum_{i=1}^{m} \frac{1}{i}}$. GenePop's

test of genic differentiation tests the null hypothesis that alleles are drawn from the same distribution at all sites.

Population differentiation was also estimated by calculating $F_{ST}$ for each locus and pairwise $F_{ST}$ (Weir and Cockerham 1984's Theta) between sites in GenePop. $F_{ST}$ describes the reduction in heterozygosity of sub-populations relative to the total heterozygosity across sites, or across both sites in pairwise comparisons. In undifferentiated populations $F_{ST} = 0$ whereas in differentiated populations $F_{ST}$ tends to 1. An analysis of molecular variance (AMOVA) using 999 permutations was performed on pairwise $F_{ST}$ in GenAlEx, using FDR correction ($m = 28$, Benjamini and Yekutieli 2001).

To visualise the genetic distance between sites, GenePop's pairwise $F_{ST}$ values were used to produce a multi-dimensional scaling (MDS) plot in XLSTAT (version 18.07, Addinsoft). $F_{ST}$ was also used to estimate the number of migrants per generation (M) between pairs of sites by the formula $M = \frac{1}{4}(\frac{1}{F_{ST}} - 1)$. To convert this to number migrants per year, M was divided by 9, the age at 50% maturity between that of females (10 years) and males (8 years) determined by Bergstad (1990). It should be noted that the tests employed by GenePop and LOSITAN assume Hardy-Weinberg equilibrium and the island model of migration proposed by Wright (1940), where sub-populations receive a constant proportion of immigrants from the overall population and the effects of migration balance those of genetic drift.

## Mantel tests: isolation by distance for abiotic and biotic variables

Isolation by distance was tested in GenAlEx using a Mantel test, which tests for a correlation between pairwise genetic distance and geographic distance matrices between sites. Values in the matrices were randomized 999 times (permutations) and each randomization was tested to generate probabilities of obtaining correlations greater than the observed correlation. Geographic distances by sea between sites were measured with the path function in Google Earth (version 7.1.5.1557). Pairwise linearized $F_{ST}$ ($F_{ST}/(1-F_{ST})$) was used for the genetic distance matrix, using pairwise $F_{ST}$ values from GenePop. It is preferable to use linearized $F_{ST}$ for isolation by distance analyses when the study area is narrow and distances between sites exceed half the width of the study area (Rousset 1997). The test was also performed using pairwise $F_{ST}$ for comparison. Mantel tests were also performed to test for correlations

with pairwise differences in bottom depth, sill depth, bottom salinity, bottom oxygen, and bottom temperature between sites (Table 1).

Mantel tests were performed to test for a correlation between genetic distances and differences in the condition indices of those individuals that were genetically sampled between sites. For these tests, we used differences in regression slopes of log-transformed weight versus log-transformed PAFL, and differences in the means of hepatosomatic index (HSI) and gonadosomatic index (GSI) between sites.


## Condition indices: length-weight regressions

For consistency across datasets all length data were rounded down to the nearest centimetre and weights were rounded down to the nearest gram. Rounded PAFLs and weights were log-transformed prior to statistical analyses. All statistical tests were performed in R (version 3.0.3, see Appendix 9 for script).

Differences in the length-total weight relationships between sites were tested using analyses of covariance (ANCOVAs), which tested for differences in the slope of length-weight between sites. Wherever significant interactions were not found between sites (i.e. the regression gradients were not significantly different), the interaction term was removed and the difference in regression intercepts was tested assuming parallel regressions. Because Trondheimsleia samples consisted of only large adults ($\geq 14$ cm PAFL), a second ANCOVA was performed while excluding Trondheimsleia samples. The Skagerrak data allowed for testing length-weight between years. Differences in length-weight were also tested between fjord sites using gutted weights and ANCOVA.


## Condition Indices: HSI and GSI

The Hepatosomatic Index (HSI) and Gonadosomatic Index (GSI) were calculated for grenadiers from Inner and Outer Lustrafjord, Masfjord, and Korsfjord (Table 2). Because body weights were taken with more precision for these samples, they were rounded down to the nearest 0.1g. HSI was calculated by $\frac{Liver\ weight}{Body\ weight} \times 100$ and GSI by $\frac{Gonad\ weight}{Body\ weight} \times 100$. Comparisons between sites were performed using Generalized Linear Models (GLMs), assuming a binomial distribution. Tukey's Honest Significant Difference (Tukey-HSD) test was performed to view pairwise site comparisons.

# Results

## Population Genetics

Gene diversity varied across loci, with the number of alleles ranging from 6 (Crup1) to 24 (CorRu3, Table 4). The number of observed heterozygotes was generally lower than expected across loci (Table 4) and populations (Table 5), which is reflected in some of the high inbreeding coefficients ($F_{IS}$) observed (average 0.1464 across all loci and populations, Table 4). Allele richness was highest in Trondheimsleia (8.116) and was lower at the other sites (between 6.087 and 6.866, Table 5).

After false discovery rate (FDR) correction, 19 out of 64 locus-site combinations were not in Hardy-Weinberg equilibrium (Appendix 6), which was much higher than the 5% that could be attributed to chance. This value was reduced to 14 after removing rare homozygotes. Deviations from Hardy-Weinberg were found at Crup1, CorRu2, CorRu3, and CorRu11, the same loci flagged by Micro-Checker at four or more sites. These four loci displayed the highest values of $F_{IS}$ (Table 4). $F_{IS}$ was low for the other four loci. Removing the four flagged loci meant no deviations from Hardy-Weinberg were found. Their removal resulted in reduced statistical power in the tests of genic differentiation and the AMOVA. While P-values were larger, significant differences were still found between most pairs of sites; only a few comparisons became non-significant after removing the four flagged loci. Therefore, accounting for rare homozygotes and null-allele loci did not significantly alter the patterns of Hardy-Weinberg equilibrium or the genetic structure patterns observed.

Table 4: Summary statistics for each microsatellite locus for *Coryphaenoides rupestris*, indicating the mean number of alleles (N), observed ($H_O$) and expected heterozygosity ($H_E$), deviation from Hardy-Weinberg equilibrium ($F_{IS}$), genetic differentiation among samples ($F_{ST}$) across sites, P-values for exact tests of genic differentiation across sites, and the grand mean across all loci and populations.

| Locus | N | $H_O$ | $H_E$ | $F_{IS}$ | $F_{ST}$ | P* |
|---|---|---|---|---|---|---|
| Crup1[#] | 6 | 0.152 | 0.256 | 0.3682 | 0.0033 | <0.001 |
| Crup6 | 12 | 0.761 | 0.775 | 0.0200 | 0.0152 | <0.001 |
| CorRu2[#] | 21 | 0.614 | 0.751 | 0.1779 | 0.0444 | <0.001 |
| CorRu3[#] | 24 | 0.418 | 0.710 | 0.4449 | 0.0703 | <0.001 |
| CorRu4 | 13 | 0.572 | 0.570 | 0.0040 | 0.0303 | <0.001 |
| CorRu11[#] | 18 | 0.657 | 0.869 | 0.2923 | 0.0173 | <0.001 |
| CorRu12 | 15 | 0.828 | 0.815 | -0.0196 | 0.0255 | <0.001 |
| CorRu33 | 17 | 0.730 | 0.704 | 0.0207 | 0.0194 | <0.001 |
| | | | | | | |
| Grand mean | 8.875 | 0.592 | 0.681 | 0.1464 | 0.0297 | <0.001 |

* Reported as '0' in GenePop.
[#] Loci flagged by Micro-Checker and Hardy-Weinberg tests.

Table 5: Summary statistics for 8 sampling sites of *Coryphaenoides rupestris*, showing average allele richness (R), observed ($H_O$) and expected ($H_E$) heterozygosity.

| Population | R | $H_O$ | $H_E$ |
|---|---|---|---|
| Korsfjorden | 6.886 | 0.614 | 0.677 |
| Inner Lustrafjorden | 6.778 | 0.578 | 0.678 |
| Outer Lustrafjorden | 6.660 | 0.556 | 0.698 |
| Masfjorden | 6.087 | 0.609 | 0.699 |
| Skagerrak 2001 | 6.159 | 0.553 | 0.640 |
| Skagerrak 2008 | 6.574 | 0.600 | 0.648 |
| Skagerrak 2016 | 6.280 | 0.633 | 0.655 |
| Trondheimsleia | 8.116 | 0.590 | 0.757 |

Exact tests of genic differentiation revealed significant population structuring across all loci and populations, with an average $F_{ST}$ of 0.0297 (P < 0.001, Table 4). Pairwise tests of genic differentiation revealed significant differences between all but six pairs of sites, even after FDR correction (P ≤ 0.001, Table 6, Appendix 7). The analysis of molecular variance (AMOVA) generated from pairwise $F_{ST}$ values calculated in GenAlEx revealed similar patterns (P ≤ 0.001, Table 7), with an additional significant difference found between Kor and SK08. Excluding the flagged loci (Crup1, CorRu2, CorRu3 and CorRu11) resulted in a loss of significance in seven pairwise comparisons for the test of genic differentiation (Appendix 7) and one pairwise comparison in the AMOVA (Lus-In and SK01). Temporal samples from the Skagerrak were homogeneous across all tests. Pairwise comparisons with Skagerrak samples may have been hindered by sample size, particularly for 2016 (17 samples). Tests of pairwise genic differentiation and AMOVA were performed on only Skagerrak samples, and the results were consistent with those in Table 6 and Table 7. Temporal Skagerrak samples were therefore pooled (sample size of 94) and tests of genic differentiation and AMOVA were performed again between sites.

Having grouped all Skagerrak samples, the test of genic differentiation still revealed significant genetic structuring overall (P < 0.001) and for each locus (P < 0.001 for all 8 loci). Testing for pairwise genic differentiation revealed highly significant differences between all pairs of sites after FDR correction, except between Skagerrak and Korsfjord (Table 8, Appendix 8). The AMOVA revealed significant differences between all pairs of sites after FDR correction (Table 9). Excluding the flagged loci had no effect on the results; all significant pairwise differences were maintained in the test of genic differentiation and the AMOVA.

Table 6: P-values for pairwise genic differentiation test in GenePop, for *Coryphaenoides rupestris* from 8 sampling sites/years. Significant P-values after correction using Benjamini-Yekutieli's false discovery rate (FDR) are in bold. Refer to Appendix 7 for full result output.

| | Kor | Lus-In | Lus-Out | Mas | SK01 | SK08 | SK16 | TL |
|---|---|---|---|---|---|---|---|---|
| Kor | - | | | | | | | |
| Lus-In | **<0.001** | - | | | | | | |
| Lus-Out | **<0.001** | **<0.001** | - | | | | | |
| Mas | **<0.001** | **<0.001** | **<0.001** | - | | | | |
| SK01 | 0.129 | **<0.001** | **<0.001** | **<0.001** | - | | | |
| SK08 | 0.141 | **<0.001** | **<0.001** | **<0.001** | 0.229 | - | | |
| SK16 | 0.284 | **<0.001** | **<0.001** | **<0.001** | 0.540 | 0.540 | - | |
| TL | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | - |

Table 7: Pairwise differentiation between sites and years for *Coryphaenoides rupestris*. $F_{ST}$ values calculated in GenePop (below diagonal) and P-values (above diagonal) from an AMOVA showing the probability of randomly assigning individuals to populations and obtaining $F_{ST}$ values greater than those observed, from 999 permutations in GenAlEx. Significant P-values after correction using Benjamini-Yekutieli's false discovery rate (FDR) are in bold.

| | Kor | Lus-In | Lus-Out | Mas | SK01 | SK08 | SK16 | TL |
|---|---|---|---|---|---|---|---|---|
| Kor | - | **0.001** | **0.001** | **0.001** | 0.011 | **0.001** | 0.112 | **0.001** |
| Lus-In | 0.0165 | - | **0.001** | **0.001** | **0.001** | **0.001** | **0.001** | **0.001** |
| Lus-Out | 0.0203 | 0.0228 | - | **0.001** | **0.001** | **0.001** | **0.001** | **0.001** |
| Mas | 0.0203 | 0.0087 | 0.0338 | - | **0.001** | **0.001** | **0.001** | **0.001** |
| SK01 | 0.0038 | 0.0299 | 0.0248 | 0.0308 | - | 0.015 | 0.110 | **0.001** |
| SK08 | 0.0062 | 0.0341 | 0.0238 | 0.0332 | -0.0009 | - | 0.434 | **0.001** |
| SK16 | -0.0027 | 0.0253 | 0.0248 | 0.0235 | -0.0021 | -0.0034 | - | **0.001** |
| TL | 0.0467 | 0.0363 | 0.0331 | 0.0393 | 0.0525 | 0.0535 | 0.0577 | - |

Table 8: P-values for pairwise genic differentiation test in GenePop, for *Coryphaenoides rupestris* from 6 sampling sites. Temporal Skagerrak samples have been pooled (SK). Significant P-values after correction using Benjamini-Yekutieli's false discovery rate (FDR) are in bold. Refer to Appendix 8 for full result output.

| | Kor | Lus-In | Lus-Out | Mas | SK | TL |
|---|---|---|---|---|---|---|
| Kor | - | | | | | |
| Lus-In | **<0.001** | - | | | | |
| Lus-Out | **<0.001** | **<0.001** | - | | | |
| Mas | **<0.001** | **<0.001** | **<0.001** | - | | |
| SK | 0.029 | **<0.001** | **<0.001** | **<0.001** | - | |
| TL | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | - |

Table 9: Pairwise differentiation between sites for *Coryphaenoides rupestris*. Temporal Skagerrak samples have been pooled (SK). $F_{ST}$ values calculated in GenePop (below diagonal) and P-values (above diagonal) from an AMOVA showing the probability of randomly assigning individuals to populations and obtaining $F_{ST}$ values greater than those observed, from 999 permutations in GenAlEx. Significant P-values after correction using Benjamini-Yekutieli's false discovery rate (FDR) are in bold.

|         | Kor    | Lus-In | Lus-Out | Mas    | SK     | TL     |
|---------|--------|--------|---------|--------|--------|--------|
| Kor     | -      | **0.001** | **0.001** | **0.001** | **0.002** | **0.001** |
| Lus-In  | 0.0165 | -      | **0.001** | **0.001** | **0.001** | **0.001** |
| Lus-Out | 0.0203 | 0.0228 | -       | **0.001** | **0.001** | **0.001** |
| Mas     | 0.0203 | 0.0087 | 0.0338  | -      | **0.001** | **0.001** |
| SK      | 0.0043 | 0.0299 | 0.0266  | 0.0318 | -      | **0.001** |
| TL      | 0.0467 | 0.0363 | 0.0331  | 0.0393 | 0.0571 | -      |
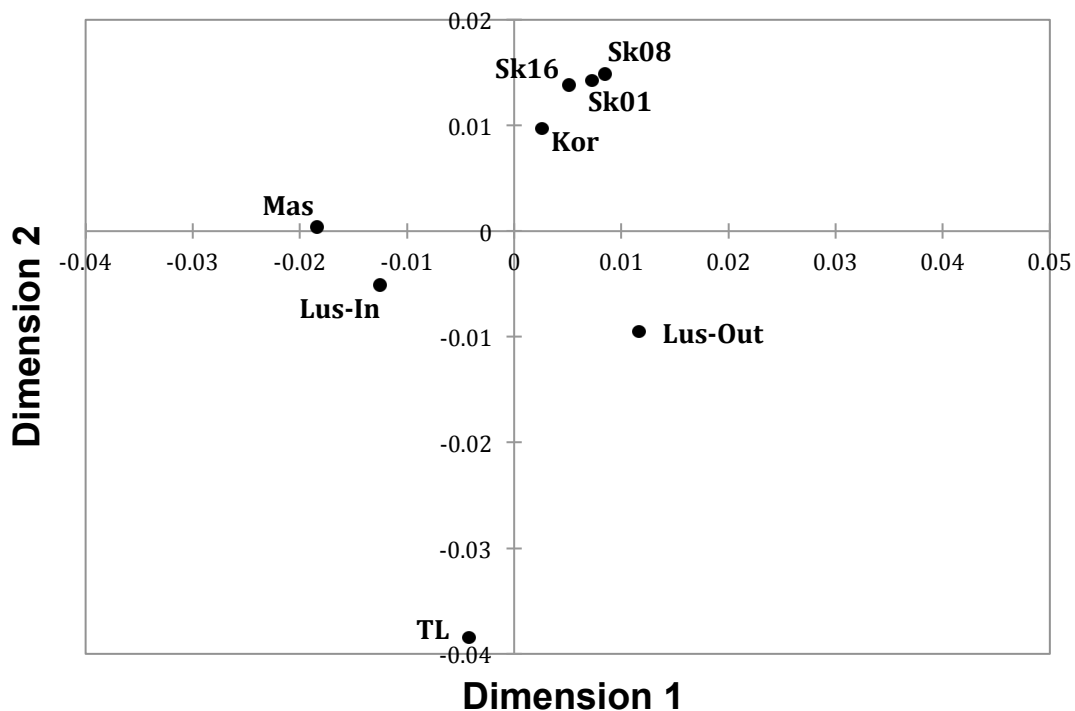


Figure 5: Multi-dimensional scaling (MDS) plot showing the genetic distance of *Coryphaenoides rupestris* between eight Norwegian sites, based on pairwise $F_{ST}$ (GenePop, Table 7).

A multi-dimensional scaling (MDS) plot (Figure 5) using pairwise $F_{ST}$ values (Table 7) reveals that Trondheimsleia grenadiers were the most genetically distant from the other sites. A tight cluster between years depicted temporal stability in the Skagerrak. Skagerrak grenadiers were more similar to Korsfjord grenadiers than other sites. Inner and Outer Lustrafjord grenadiers were genetically distant, and Inner Lustrafjord was more similar to Masfjord than to Outer Lustrafjord. The $F_{ST}$ values translated to a low number of migrants between sites, the highest being 6.4 migrants

per year between Korsfjord and Skagerrak, but less than 2 between all other pairs of sites (Table 10).

Table 10: The number of migrants between pairs of sites per generation ($M = \frac{1}{4}(\frac{1}{F_{ST}} - 1)$, above diagonal) and per year ($\frac{M}{9\ years}$, below diagonal).

|        | Kor | Lus-In | Lus-Out | Mas | SK | TL |
|--------|-----|--------|---------|-----|-----|-----|
| Kor    | -   | 14.9   | 12.1    | 12.1 | 57.9 | 5.1 |
| Lus-In | 1.7 | -      | 10.7    | 28.1 | 8.1 | 6.6 |
| Lus-Out| 1.3 | 1.2    | -       | 7.1  | 9.1 | 7.3 |
| Mas    | 1.3 | 3.2    | 0.8     | -    | 7.6 | 6.1 |
| SK     | 6.4 | 0.9    | 1.0     | 0.8  | -   | 4.1 |
| TL     | 0.6 | 0.7    | 0.8     | 0.7  | 0.5 | -   |

## Isolation by distance for abiotic and biotic variables

Results of the Mantel tests showed a significant positive correlation between linearized $F_{ST}$ and geographic distance between sites (r=0.702, P=0.001) and differences in bottom depth between sites (r=0.555, P=0.014, Table 11, Figure 6). A positive trend between genetic distance and bottom temperature was found. The trend was non-significant with linearized $F_{ST}$ (r=0.639, P=0.070), but significant using untransformed $F_{ST}$ (r=0.639, P=0.009, Table 11). The Skagerrak had the coldest bottom temperature out of all the sites (Table 1); between-site differences of 0.9 °C or greater were for all pairwise site comparisons that included the Skagerrak. No significant correlations were found between genetic distance and the other environmental variables or condition indices (Table 11, Figure 6).

Table 11: Results of Mantel tests of correlation between genetic distance (linearized $F_{ST}$ and untransformed $F_{ST}$) and differences between sites in: geographic distance, bottom depth, sill depth, bottom salinity, bottom oxygen, bottom temperature, growth, hepatosomatic index (HSI) and gonadosomatic index (GSI). Correlation factors (r) and P-values (P(random correlation ≥ observed correlation)) from 999 permutations are shown. Significant P-values are in bold.

|                                | Linearized $F_{ST}$ | | Untransformed $F_{ST}$ | |
|--------------------------------|-----|---------|-----|---------|
| Pairwise site difference       | r   | P-value | r   | P-value |
| Geographic distance (km)       | 0.702 | **0.001** | 0.701 | **0.001** |
| Bottom depth (m)               | 0.555 | **0.014** | 0.551 | **0.032** |
| Sill depth (m)                 | 0.376 | 0.133   | 0.385 | 0.149   |
| Bottom salinity (PSU)          | 0.514 | 0.167   | 0.517 | 0.173   |
| Bottom oxygen (mg/L)           | -0.065 | 0.330  | -0.074 | 0.306  |
| Bottom temperature (°C)        | 0.639 | 0.070   | 0.639 | **0.009** |
| Growth (log weight v log PAFL) | 0.629 | 0.134   | 0.622 | 0.119   |
| HSI                            | -0.153 | 0.454  | -0.146 | 0.489  |
| GSI                            | 0.604 | 0.187   | 0.601 | 0.135   |
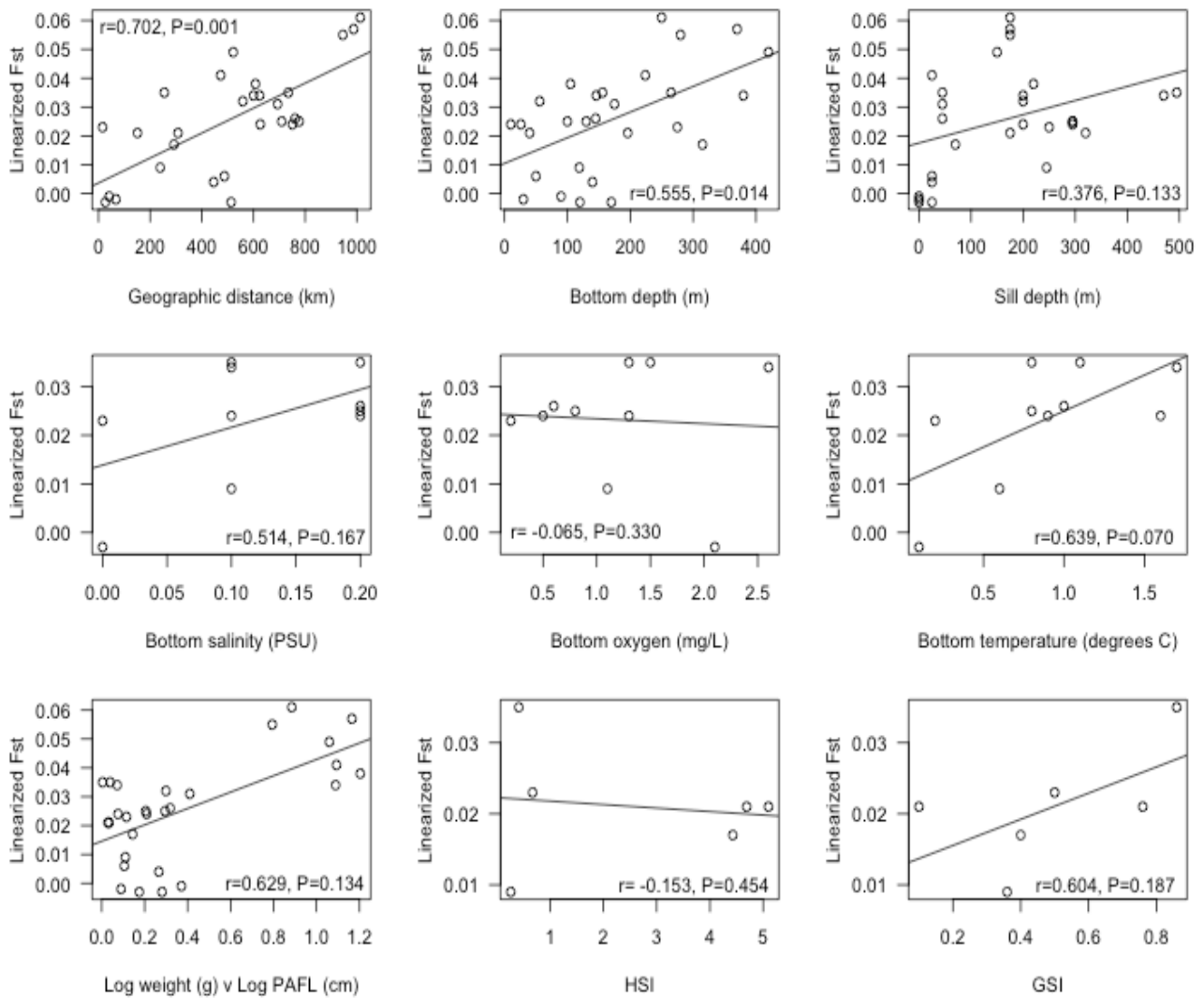
Figure 6: Pairwise genetic distance (linearized $F_{ST}$) of *Coryphaenoides rupestris* plotted against pairwise differences between sites for: geographic distance, bottom depth, sill depth, bottom salinity, bottom oxygen, bottom temperature, growth rate (log weight v log PAFL), hepatosomatic index (HSI) and gonadosomatic index (GSI). Results of the Mantel test are shown by the correlation factor (r) and P values (P(random correlation ≥ observed correlation)) following 999 permutations in GenAlEx. Three sites lacked salinity, oxygen and temperature data, resulting in 10 pairwise site comparisons for these analyses. HSI and GSI were only collected at four fjord sites.

## Condition indices: length-weight regressions

The analysis of covariance (ANCOVA) comparing all sites revealed significant differences in the relationship of log-transformed weights to log transformed PAFLs between sites (F = 8.249, df = 7, P < 0.001). The test was performed again while excluding Trondheimsleia samples, because these only consisted of individuals ≥14 cm PAFL. Differences between sites were maintained when excluding Trondheimsleia samples (F = 4.556, df = 6, P < 0.001). Pairwise significance tests

were significant regardless of whether Trondheimsleia was excluded, but exclusion did weaken P-values (Table 12). Lustrafjord grenadiers had the largest increase in weight relative to PAFL and this relationship was steeper in the inner fjord (Figure 7). Trondheimsleia grenadiers had the flattest length-weight regression, but the accuracy of this result is uncertain due to the absence of grenadiers <14 cm in this sample. No temporal differences in length-weight were found in the Skagerrak.

When comparing only the fjord sites using gutted weights, no significant differences were found in length-weight regressions overall (F = 0.299, df = 3, P = 0.826). However, there were differences in their intercepts (F = 36.586, df = 3, P < 0.001). Inner Lustrafjord and Masfjord had the highest weights-for-length while Korsfjord had the lowest (Figure 8, Table 13). There was a large difference between the mean weight and gutted weight of grenadiers from Inner Lustrafjord relative to the other fjords (Table 14), suggesting the viscera could have influenced the differences in regressions observed.

Table 12: Pairwise P-values from two analyses of covariance (ANCOVA) comparing the slopes of log-transformed weights versus log-transformed pre-anal fin lengths of *Coryphaenoides rupestris* from Norwegian sites. P-values including all sites (above diagonal) and when excluding Trondheimsleia (below diagonal) are shown, with significant values in bold.

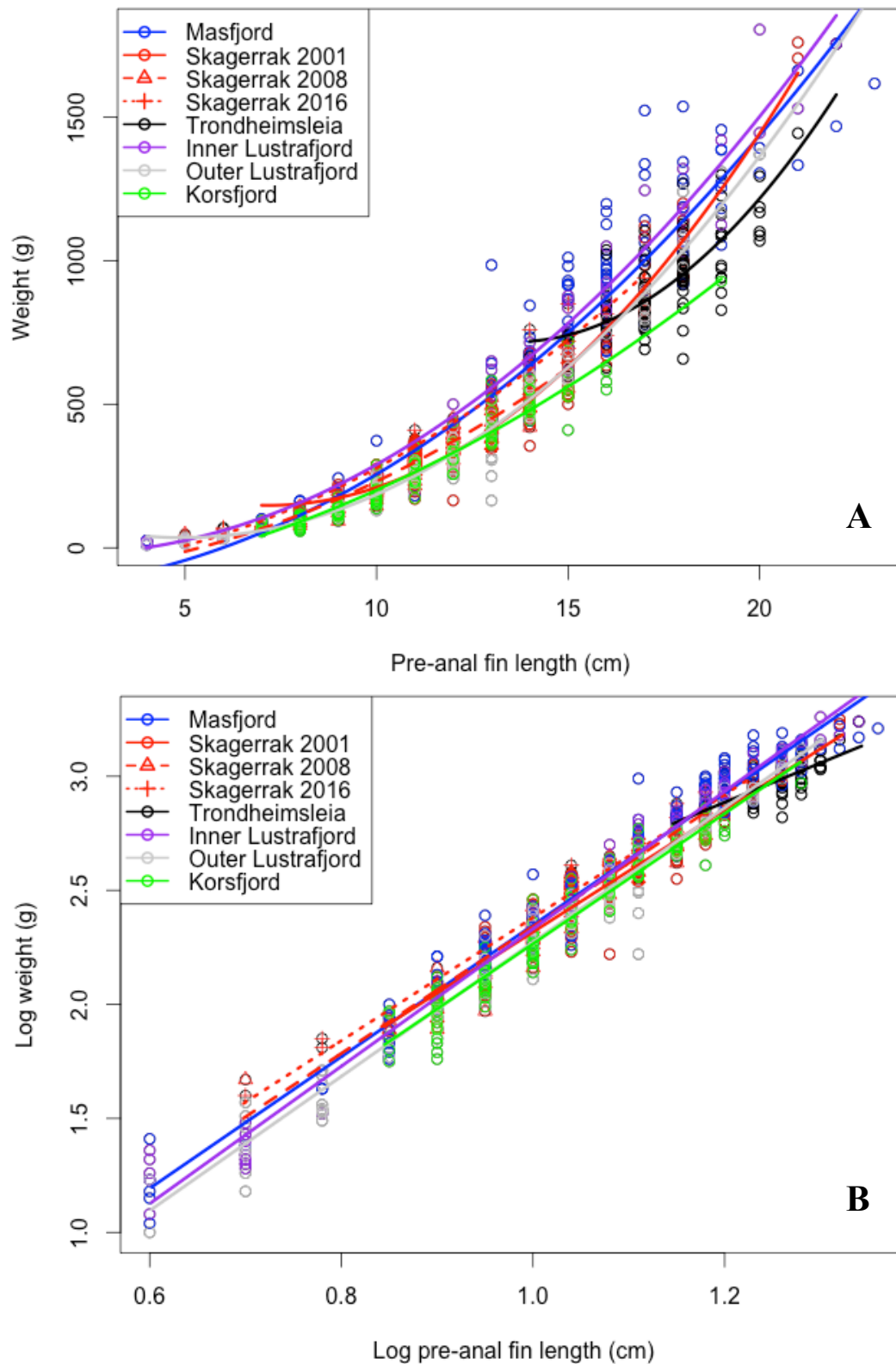| | Kor | Lus-In | Lus-Out | Mas | SK01 | SK08 | SK16 | TL |
|---|---|---|---|---|---|---|---|---|
| Kor | - | 0.080 | 0.359 | 0.816 | **0.041** | 0.471 | 0.144 | **<0.001** |
| Lus-In | 0.087 | - | 0.220 | **0.021** | **<0.001** | **0.027** | **0.004** | **<0.001** |
| Lus-Out | 0.370 | 0.231 | - | 0.283 | **<0.001** | 0.121 | **0.022** | **<0.001** |
| Mas | 0.820 | **0.024** | 0.295 | - | **0.002** | 0.305 | 0.068 | **<0.001** |
| SK01 | **0.046** | **<0.001** | **<0.001** | 0.003 | - | 0.361 | 0.986 | **<0.001** |
| SK08 | 0.481 | **0.031** | 0.130 | 0.317 | 0.371 | - | 0.472 | **<0.001** |
| SK16 | 0.154 | **0.005** | **0.026** | 0.075 | 0.987 | 0.482 | - | **<0.001** |

Figure 7: A) Weight versus pre-anal fin length and B) log-transformed weight versus log-transformed pre-anal fin length, for *Coryphaenoides rupestris* from eight Norwegian sites. Open circles show individual data. Polynomial regression was used for (A) and linear regression for (B).
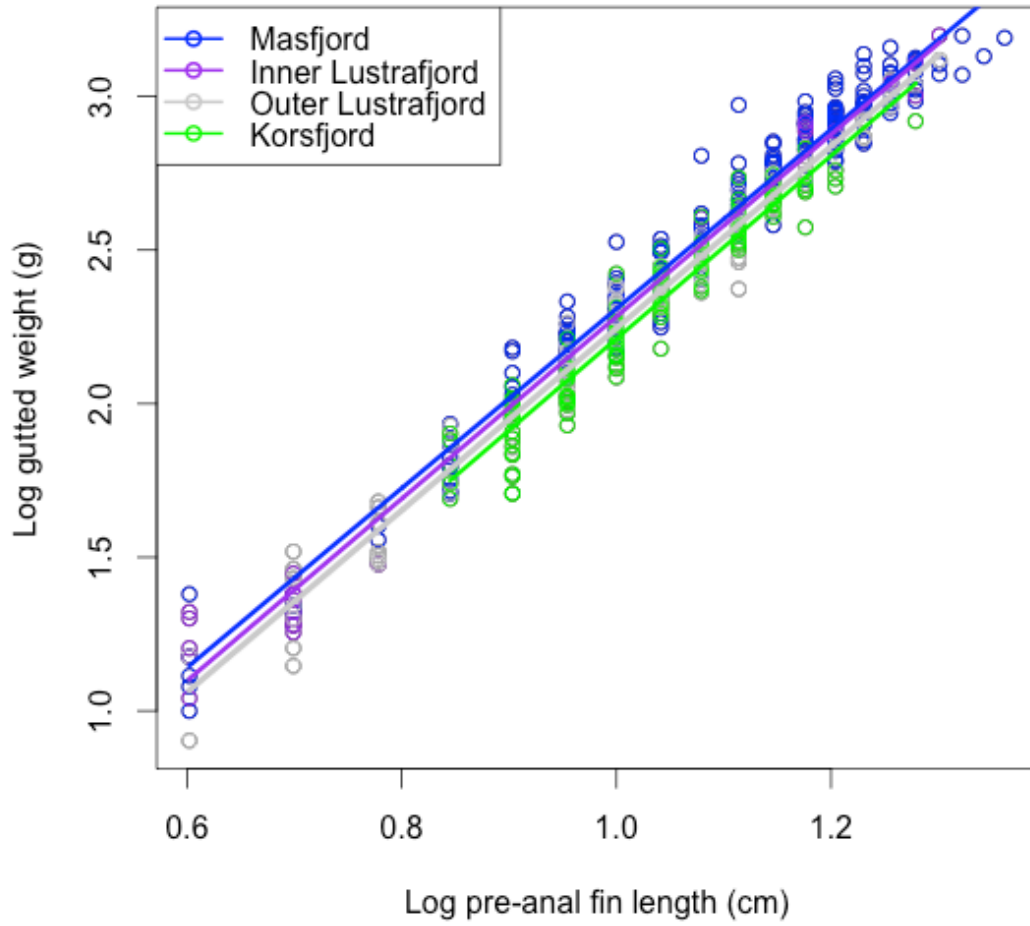
Figure 8: Log-transformed pre-anal fin lengths versus log-transformed gutted weights of *Coryphaenoides rupestris* from four Norwegian fjord sites. Open circles show individual data. A linear regression was used.

Table 13: Pairwise P-values from an analysis of covariance (ANCOVA) comparing the intercepts of linear regressions for log-transformed gutted weight versus log-transformed pre-anal fin length of *Coryphaenoides rupestris* from Norwegian fjord sites. Significant values are in bold.

|  | Kor | Lus-In | Lus-Out | Mas |
|---|---|---|---|---|
| Kor | - |  |  |  |
| Lus-In | **<0.001** | - |  |  |
| Lus-Out | **0.001** | **0.031** | - |  |
| Mas | **<0.001** | 0.128 | **<0.001** | - |

Table 14: Summary of range (top), mean (middle) and standard deviation (bottom) of biological characteristics of *Coryphaenoides rupestris* from eight Norwegian sites. NA indicates no data were available.

| | Kor | Lus-In | Lus-Out | Mas | SK01 | SK08 | SK16 | TL |
|---|---|---|---|---|---|---|---|---|
| Pre-anal fin length (cm) | 7 – 19<br>11.1<br>2.6 | 4 – 22<br>12.1<br>5.8 | 4 – 20<br>11.4<br>3.4 | 4 – 23<br>13.0<br>3.9 | 7 – 21<br>12.2<br>2.2 | 5 – 15<br>11.1<br>1.9 | 5 – 17<br>11.7<br>4.0 | 14 – 22<br>17.5<br>1.4 |
| Weight (g) | 56 – 928<br>271.8<br>183.0 | 12 – 1805<br>579.9<br>534.7 | 10 – 1370<br>320.1<br>273.0 | 11 – 1662<br>545.7<br>422.6 | 70 – 1760<br>386.8<br>226.8 | 47 – 686<br>314.0<br>135.5 | 40 – 940<br>464.7<br>328.5 | 597 – 1755<br>925.6<br>182.4 |
| Gutted weight (g) | 49 – 831<br>247.3<br>171.1 | 11 – 1580<br>369.3<br>460.7 | 8 – 1315<br>303.0<br>243.7 | 10 – 1577<br>505.9<br>391.9 | NA | NA | NA | NA |
| GSI | 0.03 – 4.36<br>0.81<br>0.99 | 0.10 – 4.68<br>1.32<br>1.13 | 0.02 – 5.60<br>0.51<br>0.81 | 0.01 – 12.18<br>1.59<br>2.12 | NA | NA | NA | NA |
| HSI | 0.90 – 11.05<br>6.10<br>2.66 | 0.32 – 6.03<br>1.82<br>1.27 | 0.15 – 4.90<br>0.99<br>0.71 | 0.22 – 7.11<br>2.11<br>1.30 | NA | NA | NA | NA |

## Condition Indices: HSI and GSI

Significant differences between sites for grenadier condition in terms of HSI ($F$ = 252.48, df = 3, $P < 0.001$) and GSI ($F$ = 15.91, df = 3, $P < 0.001$) were found. Mean HSI was much higher in Korsfjord compared to the other sites, while Outer Lustrafjord grenadiers had the lowest mean values for both HSI and GSI (Tables 14, 15). The lower mean HSI and GSI of Outer Lustrafjord compared to Inner Lustrafjord show differences in condition occurring within Lustrafjord.

Table 15: P-values from a Generalized Linear Model (GLM) comparing *Coryphaenoides rupestris* condition from 4 Norwegian fjord sites using Hepatosomatic Index (above diagonal) and Gonadosomatic Index (below diagonal). Significant values are in bold.

| | Kor | Lus-In | Lus-Out | Mas |
|---|---|---|---|---|
| Kor | - | **<0.001** | **<0.001** | **<0.001** |
| Lus-In | 0.271 | - | **<0.001** | 0.462 |
| Lus-Out | 0.212 | **0.003** | - | **<0.001** |
| Mas | **0.001** | 0.850 | **<0.001** | - |

# Discussion

This study was the first to investigate the population structure of roundnose grenadier from Norwegian fjords. Microsatellite DNA analyses show strong evidence for spatial population genetic structure in southwestern Norway, with limited gene flow between populations. Furthermore, temporal stability exists in the Skagerrak. The data suggest isolation by geographical distance and bathymetric barriers as the likely forces of spatial population structuring. Evidence of small-scale population structuring was also found, suggesting two sub-populations occur within Lustrafjord. Results of morphological, hepatosomatic (HSI) and gonadosomatic (GSI) indices reveal differences in the body conditions of grenadiers among the sites sampled, indicating possible differences in environmental conditions between sites.

## Spatial and temporal population structure

Genetic analyses using eight microsatellite markers reveal characteristic evidence for sub-divided populations: limited gene flow between populations, high inbreeding coefficients ($F_{IS}$) and a deficiency of heterozygotes leading to deviations from Hardy-Weinberg equilibrium. Allele richness and diversity is low relative to North Atlantic populations (Knutsen et al. 2012; White et al. 2010), especially for fjord and Skagerrak sub-populations. This indicates a post-glacial (re)colonization to these marine areas marked by a genetic bottleneck or founder effect, a pattern that has been observed in other species in Northern Europe (Gonzalez et al. 2016; Francisco et al. 2009).

The Trondheimsleia population of roundnose grenadier is the most isolated among the sites studied. Trondheimsleia grenadiers were the most distant from all other sites in tests of genic differentiation and genetic distance, with fewer than eight migrants per generation (<1 per year) estimated with each of the fjords and the Skagerrak to the south (Table 10). Average allele richness is the highest in Trondheimsleia (Table 5). This suggests that this population may have been established before fjord and Skagerrak sub-populations. Trondheimsleia has a closer proximity to the Norwegian continental slope than the fjords and the Skagerrak, with fewer bathymetric barriers between it and the North Atlantic populations from where they most likely originated. Historic gene flow might therefore have had more impact between Trondheimsleia and North Atlantic grenadiers. Samples from

Trondheimsleia were the same as those sampled by Knutsen et al. (2012), who found Trondheimsleia grenadiers to be strongly isolated from Skagerrak and North Atlantic populations. The present study supports and expands on these findings by additionally suggesting they are distinct from Norwegian fjord populations. The findings of the Mantel tests (Table 11, Figure 6) suggest geographic distance and depth might present dispersal barriers to and from Trondheimsleia. Trondheimsleia is the shallowest and most distant of the sites geographically.

Results of genic differentiation and genetic distance suggest grenadiers from Inner and Outer Lustrafjord are distinct from all other sites. Lustrafjord is one of Norway's most isolated fjords, situated 170 km within Norway's longest (200 km) and deepest (1300 m) fjord. The deep Sognefjord might act as a barrier to movement between the smaller fjord branches. This migratory barrier has been observed with other species in Sognefjord. A sub-population of herring is known to occur in Lustrafjord, with some migratory movement between Lustrafjord and other fjord branches within Sognefjord (Aasen 1952). The results also provide evidence of within-fjord population structuring, with Inner and Outer Lustrafjord sub-populations differing significantly from each other. The proximity of these sites, located approximately 16 km apart, goes against the trend of isolation by geographic distance found. The strong genetic structuring observed in grenadiers within Lustrafjord provides evidence of structuring by depth; Inner Lustrafjord is located at 375 m and Outer Lustrafjord at 650 m. Although the bathymetry of the fjord does not prevent movement within the fjord for the pelagic herring (Aasen 1952), the 320 m sill between the inner and outer fjord basins might be a barrier to adult grenadier movement (Figure 2C). A circulation model by the Institute of Marine Research (IMR) revealed water movement over the sill separating the Inner and Outer Lustrafjord basins is very slow (<10 cm/sec) and limited (Lars Asplin, pers. comm. 2017). The bathymetry of Lustrafjord and differences in depth provide the most plausible explanation for population structuring in Lustrafjord grenadiers. Lustrafjord is relatively understudied, but on-going investigations suggest it contains a unique species assemblage, particularly in the benthic environment (Henrik Glenner, pers. comm. 2016). These observations and our findings suggest a unique and highly isolated environment might be present Lustrafjord. Investigating population structure of grenadiers and other species in Sognefjord and its branches could answer further questions regarding connectivity in this large fjord system.

Sample collection in Lustrafjord revealed unusually long outer pelvic fin rays (Figure 3B) on these grenadiers. These were sometimes more than twice the head length, whereas the species description describes them as "almost equal to head length" (FAO 2016). The same feature was observed in some grenadiers from Korsfjord. Unfortunately, the observation was made towards the end of the field period, so the trait was not sought after or quantified among the sites. Grenadiers can be considerably damaged during trawling and structures like their tails and fins could be lost during sampling, making such observations difficult. It would be worth investigating whether this and other potential morphological features are unique to fjord populations of grenadiers.

Population genetic analyses provided strong evidence of a sub-population occurring in Masfjord. Masfjord is a very isolated fjord, located within the larger Fensfjord. In addition, it has the shallowest sill (75 m, Figure 2D) among the sites studied. This sill is believed to be a driver of population structuring in Masfjord, even for mesopelagic fishes that occur higher in the water column (Suneetha and Nævdal 2001; Suneetha and Salvanes 2001; Kristoffersen and Salvanes 2009). Although the data do not show a significant correlation between sill depth and genetic distance, a shallow sill might represent a dispersal barrier, particularly to deep-water organisms like roundnose grenadier. The number of sampling sites might not have been enough to detect a correlation between genetic distance and sill depth. Interestingly, a lower degree of differentiation was found between Masfjord and Inner Lustrafjord than between Inner and Outer Lustrafjord, based on $F_{ST}$ (Table 7, Figure 5). One can only hypothesise as to the reason for similarity between Masfjord and Inner Lustrafjord grenadiers. This may be due to chance with the genetic markers we used. Expanding the number or markers or using different markers might reveal greater differences between these populations. Alternatively, the similarity might have resulted from a single migration or recruitment event between these localities, or as an artefact of the smaller sample size from Inner Lustrafjord.

Korsfjord grenadiers were also found to be a distinct sub-population based on genetic analysis. Interestingly, Korsfjord grenadiers are more closely related to Skagerrak grenadiers, and the number of estimated migrants between these sites was the highest observed (58 per generation, or 6.4 per year). Although still isolated within Norway's coastline and by a sill, Korsfjord is situated much closer to the coast than the other fjords (Figure 2A, 2E). Furthermore, Korsfjord's basin waters are

regularly flushed by the Norwegian Coastal Current (NCC, Figure 2A), maintaining oceanic conditions in these waters (Bakke and Sands 1977; Matthews and Sands 1973). Dispersal of pelagic eggs and larvae is therefore more likely to occur between oceanic populations of grenadier, like the Skagerrak, and Korsfjord via the NCC. Isolation by bottom depth also matches these findings, with Korsfjord and the Skagerrak having similarly deep basins relative the other sites (apart from Outer Lustrafjord). The depth and abiotic conditions in Korsfjord and the Skagerrak might therefore provide similar environments for grenadiers at these two sites.

Genetic analyses of Skagerrak samples supports the existing evidence of a sub-population and of temporal stability within this sub-population (Bergstad 1990; Longmore et al. 2010; Longmore et al. 2011; Knutsen et al. 2012). These studies propose this population is isolated by the shallow sea floor surrounding the deep Skagerrak basin, creating a barrier to movement, and by egg and larvae retention by the Skagerrak's cyclonic circulation. Samples in this study were from the same collection as those used by Knutsen et al. (2012). The present findings support previous results and provide evidence of temporal stability through 2016. Between-year comparisons in the Skagerrak were somewhat limited by sample size, especially for the 2016 samples (17 individuals).

## Isolating mechanisms

Geographic distance between sites and bathymetric features appear to be the primary drivers of population sub-division in roundnose grenadier along the Norwegian coast. Genetic distance between sub-populations was significantly correlated to isolation by distance and bottom depth differences between sites. Depth has been identified as a possible source of speciation in the *Coryphaenoides* genus (Gaither et al. 2016) and sub-division in *C. rupestris* (White et al. 2010), although the dividing boundaries there were considerably deeper (4000 m and 1200 m in both studies, respectively) than the present study's depth range (270-700 m).

Adult roundnose grenadiers are thought to be poor swimmers and relatively sedentary (Longmore et al. 2010). Although they are long-lived and may travel long distances over a lifetime, their migratory movements as adults are not well known. The long distances (up to 1000 km between Skagerrak and Trondheimsleia) between the studied sites and the complex bathymetry separating them could present obstacles

to adult grenadiers. Bathymetric barriers and ocean circulation patterns resulting from these barriers may limit dispersal in roundnose grenadier. This is exemplified by the Lustrafjord sub-populations, which were highly structured despite their geographic proximity. The sub-polar front passing through the Charlie-Gibbs Fracture Zone on the MAR has been proposed to act as a dispersal barrier for sub-populations on either side (White et al. 2010). The Skagerrak and Lustrafjord bathymetry and circulation patterns might similarly act as dispersal barriers. Roundnose grenadiers are found deeper than 180 m (FAO 2016) and are not typically found at shallower depths. Therefore, grenadiers are unlikely to overcome the shallow features or sills in Trondheimsleia and Masfjord, which are above this depth (Table 1), despite performing diel vertical feeding migrations.

Dispersal is more likely to occur via ocean currents during the long pelagic egg, larval, and juvenile phases, where they have been found as shallow as 150 m and which can last up to 7 months (Bergstad and Gordon 1994; Knutsen et al. 2012). This should allow long-range dispersal along the NCC and for dispersal in and out of the fjords despite the sills. However, these barriers appear to restrict this movement. Sills might cause the retention of offspring within fjords, as has been found in cod (Knutsen et al. 2007b). Grenadiers also appear to undergo short-range dispersal independent of ocean currents, rather than the common assumption of long-range dispersal via ocean currents (Knutsen et al. 2012). Recruitment of roundnose grenadier has been slow and episodic in Norwegian waters (Hansen et al. 2015; Bergstad et al. 2014). For dispersal between the fjords and the NCC, the timing of these early life stages may need to coincide with the advection of pelagic water layers above sill height in and out of the fjords, which depend on atmospheric circulation patterns along the Norwegian coast (Asplin et al. 1999). Dispersal might also depend on the flushing of deep fjord-basin waters, which occurs episodically when the density of water flowing over the sill exceeds that of deep basin water, the densest water in a fjord (Mann and Lazier 2006). The frequency of basin flushing varies between fjords; it can occur seasonally in Korsfjord (Bakke and Sands 1977) or approximately every eight years in Sognefjord (Svendsen 2006). Norway's coastal geography, bathymetry and circulation patterns might therefore limit gene flow during all life stages of roundnose grenadier. Egg and larval studies of roundnose grenadier within fjord systems should be performed and linked to fjord circulation models to investigate this mechanism.

The results do not provide strong evidence of any correlation between genetic distance and environmental differences between sites. No correlations were found with bottom oxygen or bottom salinity. A positive correlation between genetic distance and bottom temperature was non-significant with linearized $F_{ST}$ and significant with untransformed $F_{ST}$ (Table 11, Figure 6). Seasonal differences in sampling may have influenced these results; the Skagerrak was sampled in the winter and the fjords in the summer and autumn. However, bottom temperatures generally vary by less than 1°C seasonally at the sampled depths (Syvitsky et al. 1987), corresponding approximately to the pairwise differences observed with the Skagerrak sites. The trend between genetic distance and bottom temperature might therefore be due to chance with the sampling design. CTD data were lacking from Korsfjord and Trondheimsleia, further limiting the analysis. Despite this, deep Skagerrak waters were colder than in Lustrafjord or Masfjord, while Lustrafjord had cooler waters than Masfjord, probably due to glacial meltwater in Lustrafjord (Table 1). The nature of the sampling design is such that any correlation between genetic distances and abiotic variables may not have been detected. This could be addressed in future studies with an increase in the number of study sites.

Differences in condition indices indicate favourable conditions in Inner Lustrafjord and in Masfjord relative to the other sites. However, data was limited to morphological measurements for Trondheimsleia and Skagerrak samples. Grenadiers from both Inner Lustrafjord and Masfjord are generally 'fatter' for a given PAFL than in other fjords (Figure 8, Table 13), and the increase in weight with increasing PAFL is more rapid for Inner Lustrafjord grenadiers (Figure 7, Table 12). Grenadiers from Inner Lustrafjord and Masfjord also have the highest mean GSI, suggesting higher allocation of energy to reproductive activity. In contrast, Outer Lustrafjord grenadiers have the lowest mean GSI and HSI, and are 'thinner' for a given PAFL, indicating poor conditions at this site relative to the inner fjord. While Korsfjord grenadiers are the 'thinnest' among the fjord sites and have a relatively low mean GSI, their HSI is very high, indicating significant allocation of resources to energy storage. This might be due to food availability being higher in Korsfjord as a result of its greater proximity to the productive North Sea. Morphological indices of length-weight have previously been used to identify sub-populations of roundnose grenadier. Vinnichenko and Khlivnoi (2007) found slower growth rates in grenadiers inhabiting colder waters, but this trend was not found in the present study. The present results

appear to follow Savvatimsky and Atkinson (1993)'s claim that length-weight comparisons are influenced by feeding activity and gonad and liver weights. For example, differences in the steepness of length-weight slopes between fjord sites were lost when comparing length-gutted weight. The results indicate that there are differences in the condition of grenadiers between sites, but these cannot be attributed to differences in environmental conditions between sites based on our results.

Population structuring has also been found in tusk (Knutsen et al. 2009) and Greenland halibut (Knutsen et al. 2007a), two species with similar life stages to roundnose grenadier, marked by early pelagic stages and benthic adult stages. The findings of these studies also indicate bathymetry, ocean circulation, and geographic distance as potential drivers of population sub-division in these fishes.

## Limitations and sources of error

The primary concern in the present study was the nature of the sampling design. Roundnose grenadier are rare in many locations and the logistical arrangements regarding participation on research cruises meant that sample collection was opportunistic. The risk of a violation of random sampling was present. For example, all 108 Korsfjord grenadiers were collected from a single bottom trawl in a localised area. These may be more genetically related than if samples had been collected from several locations within this fjord. The trawl used at Korsfjord was also smaller (~12 m opening) than at Lustrafjord and Masfjord (~60 m opening), which may have led to gear avoidance behaviour by larger grenadiers. In addition, reliance on samples and datasets from multiple researchers (IMR, BIO310 masters students, and myself) created inconsistencies in the data; biological data were only collected from fjord grenadiers, PAFLs and weights were recorded to different levels of precision (e.g. 1 cm or 0.5 cm), and physical data were not collected at all sites. The cost of microsatellite work meant the sample size for the genetic work was limited for Inner and Outer Lustrafjord and temporal analyses in the Skagerrak. However, the number of loci and sample sizes used allowed for high statistical power (Ryman et al. 2006), apart from Skagerrak 2016 that was limited to 17 individuals.

The analyses assumed Hardy-Weinberg equilibrium and an island model of migration. The nature of the study area and the high level of isolation of each population resulted in the violation of these assumptions, marked by high genetic drift

relative to migratory forces. While the statistical methods were not optimal for such a case, they were still able to provide strong evidence for the structural patterns observed.

Genotype checking software (Micro-Checker, Lositan) flagged several of the microsatellite markers used in this study. Hardy-Weinberg equilibrium was violated at these loci. Although this is expected from populations with high levels of sub-division (Halvor Knutsen, pers. comm. 2017), it could also indicate a technical error during the genetic data collection procedure. The success rate when scoring loci was relatively low (Appendix 5) as a result of errors such as weak or missing allele peaks, which could have resulted from errors during DNA extraction or PCR. The primers used have only recently been developed and seldom been used in population genetic studies of the species. In spite of this, the protocol was sufficiently optimized to allow for a large sample size collected in a cost-effective manner.

Precautions were taken to minimize potential technical errors. Individuals with more than 3 non-scored loci were removed from analyses. Homozygotes for rare alleles were removed to test whether these influenced tests of Hardy-Weinberg equilibrium. Tests were performed with and without the flagged loci to test for any differences in results. The population structuring patterns observed were maintained in each case. Therefore, the flagging of loci by genotype-checking software was likely a result of true population structuring in roundnose grenadier, rather than the fault of a technical error.

## Implications for the future

The results of the study indicate roundnose grenadier exist in highly isolated sub-populations among the western Norwegian fjords studied. It is possible population sub-division also occurs in other fjords and heterogeneous environments across its distribution range. The results suggest that the fjords may support the entire life cycle of the sub-populations identified. The low level of genetic diversity relative to Trondheimsleia and oceanic populations suggest grenadiers may have settled from the North Atlantic into the fjords and the Skagerrak relatively recently, sometime after glaciers began retreating at the last glacial maximum 17,000 years ago (Syvitsky et al. 1987). A founder effect or bottleneck may have occurred, after which these groups became isolated by the complex bathymetry of the region, resulting in higher levels of

inbreeding, lower genetic diversity, and high levels of homozygosity. The poor swimming ability of adult grenadiers means they are unlikely to migrate long distances, while their long pelagic early-life stages allow some, albeit limited, dispersal between these sites.

In terms of fisheries management and conservation practices, a precautionary approach should be adopted. Roundnose grenadiers are long-lived, slow growing, and generation times are long. Overfishing has resulted in the depletion of the species in the Skagerrak (Hansen et al. 2015) and in several regions of the North Atlantic (ICES 2014; Pawlowski and Lorance 2009; NAFO 2015, Appendix 1), worsened by the slow recovery ability of this species (ICES 2014; Baker et al. 2009). As a result, roundnose grenadier has been listed as critically endangered (Iwamoto 2015). Based on the genetic structure patterns found, roundnose grenadiers from each of the fjords should be considered for management as separate stocks. Given the low gene flow observed among the sites studied here, neighbouring populations cannot be expected to replenish these areas in the short-term, if at all. Rather, recovery dependent on neighbouring sites may occur on a scale of decades or longer. The life cycle of the species is poorly understood. Should industry wish to target roundnose grenadier in Norwegian waters, careful estimates of its biomass and recruitment patterns must be performed. At present, any landings of roundnose grenadier, whether targeted or as bycatch, should be closely monitored.

Future research should incorporate other tools in the population biology toolkit; additional molecular markers, otolith microchemistry and chronology, and egg and larvae surveys would provide further insights into roundnose grenadier biology. Including samples from other sites along Norway's coast, or within large fjord systems such as Sognefjord, could reveal whether sub-populations also occur there and the structuring mechanisms involved.

## Conclusion

The present study was the first to investigate population structure in Norwegian fjords for roundnose grenadier. Strong evidence of population sub-division was found in the region, revealing the presence of isolated sub-populations in fjord habitats, and that dispersal is limited between fjord and oceanic populations. The results also support the established occurrence of sub-populations in Trondheimsleia

and the Skagerrak. Stock assessments and conservation efforts should consider each fjord population on a case-by-case basis. The species' conservation and data-deficient status stress the importance of studies on their population biology. Furthermore, their role in deep fjord ecosystems is not fully known. Further investigations into roundnose grenadier biology will help in managing their stocks in these deep-sea habitats.

# Acknowledgements

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# Appendices

Appendix 1: Summary of the *Coryphaenoides rupestris* fishery and stock status
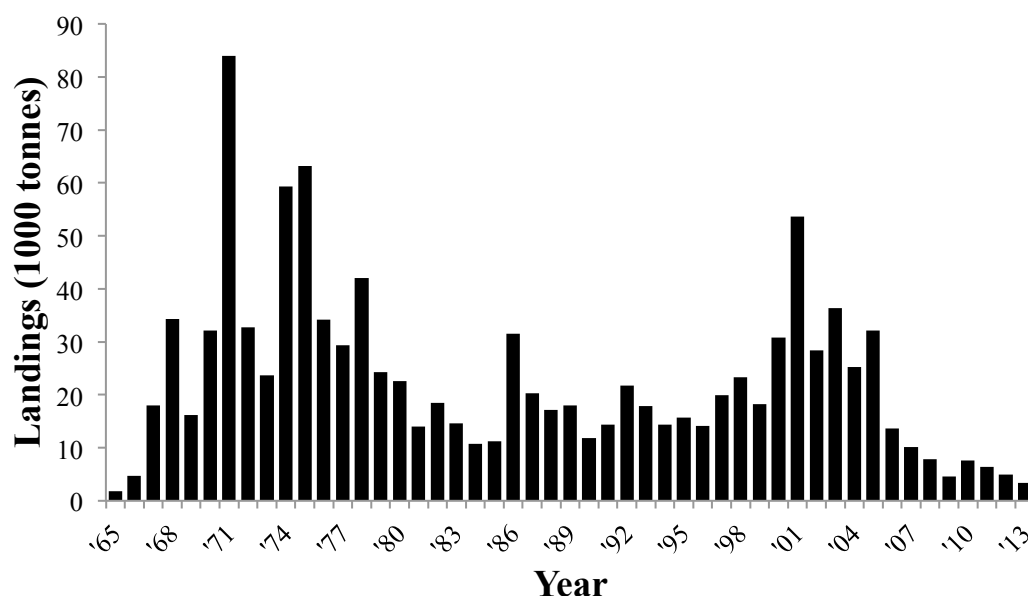


Figure 1A: Global landings of *Coryphaenoides rupestris* from 1965 to 2013. Data obtained from FAO (2016).

After the *Coryphaenoides rupestris* fishery began in 1965, landings peaked in 1971 and then declined (FAO 2016, Figure 1A). The depletion of *C. rupestris* due to fishing has been observed across the North Atlantic. The strongest fishery, along the MAR, saw landings peak at 30000 t in 1975 and since decrease to near zero in 2010 before picking up again in 2011 (ICES 2014). A dramatic decline was also seen in Canadian waters, with a 99.6% decline in relative abundance between 1978 and 2003 (Devine et al. 2006). This has resulted in the establishment of moratoriums, mesh-size regulations (NAFO 2015; Power 1999), and the species' listing as endangered in Canadian waters (COSEWIC 2009). The northeast Atlantic stock has also experienced a large decline in catches and in estimated biomass. Of major concern has been the high discard rate in northeast Atlantic fisheries, with up to 47% of individuals (28% by mass) being discarded; this has reduced due to fishery-management in recent years (ICES 2014; Pawlowski and Lorance 2009).

Catches have been comparatively low in Norwegian waters, and mostly due to by-catch from Norwegian and Scottish deep-water mixed and shrimp fisheries. The combined catches in the Barents Sea, Norwegian Sea and North Sea have mostly remained below 50 t per year since the fishery started in 1990, and ICES advises a

total allowable catch (TAC) of only 120 t per year until the stock is shown to be sustainable (ICES 2014). In the Skagerrak, the combination of intensive fishing with the episodic and slow recruitment pattern of *C. rupestris* has depleted the stock. A strong Danish fishery developed in the Skagerrak during the 1980s and peaked at around 12000 t in 2005, after which a moratorium was set (ICES 2014; Hansen et al. 2015). The stock biomass and abundance was the lowest on record in 2015 (Hansen et al. 2015). Directed local fisheries in Norway's fjords also exist, however *C. rupestris* stock assessments are not performed there (Bergstad, pers. comm. November 2015).

Fishery dependent and independent data has revealed the following patterns across most of the North Atlantic: overfishing has depleted *C. rupestris* abundance, the populations have become increasingly dominated by younger and smaller individuals (Pawlowski and Lorance 2009; Bergstad et al. 2014; Hansen et al. 2015), and long recovery times are predicted (Baker et al. 2009; ICES 2014). While fishing activity has decreased and moratoriums have been established, by-catch still occurs in all areas. Even as by-catch, their survival rate is low due to the expansion of the swim bladder and/or changes in temperature upon hauling (Devine et al. 2012). *Coryphaenoides rupestris* has qualified as 'critically endangered' since 2015 on IUCN's Red List of Threatened Species (Iwamoto 2015).

Appendix 2: Microsatellite primers developed for *Coryphaenoides rupestris* (Knutsen et al. 2008; Schneider et al. 2009; White et al. 2009). The 8 loci used in this study are in bold. Fluorescent markers for the primers ordered are written at the start of the forward sequence. Crup8 and CorRu7 were tested and dropped from the study.

| Locus | Genbank code | Repeat motif (N) | Primer sequences (5'-3') | Size range (bp) | Number of alleles |
|---|---|---|---|---|---|
| **Crup 1** | EU341322 | (TC)6 | F: NED-ATCTATCACCGCTATAAACGAAGAG<br>R: ATGCAGACGCACGGACAG | 102–118 | 12 |
| **Crup 6** | EU341327 | (AC)8 | F: VIC-CCTGGTCGATGTCCCTAC<br>R: CTGATCGTCCAGACCAGC | 179–211 | 13 |
| **CorRu2** | FJ374670.1 | (CA)11 | F: NED-TCGCTGTCCACCACCACG<br>R: TTGTTTAATGTGCTCGGTGC | 126–198 | 31 |
| **CorRu3** | FJ374671.1 | (GACA)13 | F: FAM-TGATTCCTGCTCATGCCGT<br>R: CTGGGGATATTGCGGTGAT | 84–152 | 16 |
| **CorRu4** | FJ374672.1 | (TG)10 nucs (TG)9 nucs (TG)4 nucs (TG)5 nucs (TG)5 nucs (TG)4 nucs (TG)3 | F: VIC-CAGGAAGGGAGACGGTGTGA<br>R: CACGCACATGTACACATGAG | 109–181 | 16 |
| **CorRu11** | FJ374674.1 | (TG)4 CG (TG)2 CG (TG)5 | F: NED-CATCTGCCTGATGGCCTAAT<br>R: CGTTGGCAAGGTCAAATTCT | 154–210 | 26 |
| **CorRu12** | FJ374675.1 | (CA)6 nucs (CA)3 nucs (CA)10 | F: FAM-TATGACAACACACGCACACG<br>R: TGCACATGTCTTTTGCTTCC | 174–246 | 21 |
| **CorRu33** | FJ374677.1 | (AC)19 nucs (AC)5 nucs (AC)3 | F: FAM-GGCACAGGAGTTTACAGTTTCC<br>R: AACATGCATTTGCGTCTCAG | 139–179 | 18 |
| Crup8 | EU341329 | (AC)3GC(AC)9 | F: PET-ACAGCCAAGCATGCACTG<br>R: GTCGGAGTGATTTGTCGC | 79–113 | 16 |
| CorRu7 | FJ374673.1 | (CA)12 | F: PET-CGGCTGGAAAGAAAGATGTC<br>R: TGGGAATGACCCCACACTAT | 168–312 | 36 |
| Crup2 | EU341323 | (CA)10 | F: CATGGGAGGTCCTGTGTTTC<br>R: CGTCAGTCGTATGGACTTGG | 226–228 | 2 |
| Crup3 | EU341324 | (TC)7 N (TC)8 N (TC)5 N (TC)7 N (TC)5 N (TC)3 N (TC)3 N (TC)2 N (TC)8 N (TC)5 N (CA)3 N (TA)2(CA)3 | F: ACAACACTCGCTGGGACAAC<br>R: AGGGTGGTGATGAGAAGTGAG | 216–334 | 36 |
| Crup4 | EU341325 | (CA)2 N (CA)2 N (CA)3 N (CA)2 N (CA)3 N (CA)2 N (CA)2 N (CA)4 N (CA)3 N (CA)2 N (CA)2 N (CA)4 N (CA)2 N (CA)2 | F: CCGTAGATGACACCGCATAAC<br>R: GGAAACAGCCACTTCTCCTG | 234–294 | 29 |
| Crup5 | EU341326 | (AC)8GC(AC)28 | F: GCAAACAGGTATGATGGT<br>R: GAAAACAAAATGCAAGGG | 222–390 | 61 |
| Crup7 | EU341328 | (AC)8 | F: CCCCACAATGCTCGGAAG<br>R: CTTCCCAGCACGGAGTGG | 116–136 | 9 |
| CaraA109 | FJ694864 | (CA)10N(AAAC)7 | F: CCTTGCCTCACACCTCTG<br>R: CCGTTCTTGCTTCTGTATGC | 188–202 | 4 |
| CaraC7 | FJ694869 | (TACA)11N(CA)14 | F: GCTGGTGGTCAAGTGAATC<br>R: GCATTGGCTGTATTGTGC | 132–170 | 10 |
| CorRu1 | FJ374669.1 | (TG)8 nucs (TG)4 nucs (TG)5 nucs TG)18 nucs (TG)4 | F: CTCTGTGGGAGTGGAGTGCA<br>R: CTCAGTGTGGCAGTCATCGC | 122–224 | 42 |
| CorRu28 | FJ374676.1 | (TG)9 nucs (TG)6 nucs (TG)9 | F: CAGAATCTGAAGGACTTTGG<br>R: GTACACGCATGCACATGGAC | 190–232 | 14 |

Appendix 3: Five master mixes used in this study, using a standard TaKaRa Ex HS kit. 'Multi' indicates those mixes that were diluted together for sequencing.

| | Reagent | µL per reaction |
|---|---|---|
| **Master mix 1** | x10 buffer | 2.5 |
| **(multi 1)** | Crup6-F (10 µmol) | 1 |
| | Crup6-R (10 µmol) | 1 |
| | CorRu11-F (10 µmol) | 1 |
| | CorRu11-R (10 µmol) | 1 |
| | CorRu33-F (10 µmol) | 1 |
| | CorRu33-R (10 µmol) | 1 |
| | dNTP (2.5 mmol) | 2 |
| | taq (5 U/µL) | 0.15 |
| | $ddH_2O$ (µL) | 13.35 |
| | Total (µL) | 24 |
| **Master mix 2** | x10 buffer | 2.5 |
| **(multi 2)** | CorRu2-F (10 µmol) | 1 |
| | CorRu2-R (10 µmol) | 1 |
| | CorRu3-F (10 µmol) | 1 |
| | CorRu3-R (10 µmol) | 1 |
| | dNTP (2.5 mmol) | 2 |
| | taq (5 U/µL) | 0.15 |
| | $ddH_2O$ (µL) | 15.35 |
| | Total (µL) | 24 |
| **Master mix 3** | x10 buffer | 2.5 |
| **(multi 2)** | Crup1-F (10 µmol) | 1 |
| | Crup1-R (10 µmol) | 1 |
| | dNTP (2.5 mmol) | 2 |
| | taq (5 U/µL) | 0.15 |
| | $ddH_2O$ (µL) | 17.35 |
| | Total (µL) | 24 |
| **Master mix 4** | x10 buffer | 2.5 |
| **(multi 2)** | CorRu4-F (10 µmol) | 1 |
| | CorRu4-R (10 µmol) | 1 |
| | dNTP (2.5 mmol) | 2 |
| | taq (5 U/µL) | 0.15 |
| | $ddH_2O$ (µL) | 17.35 |
| | Total (µL) | 24 |
| **Master mix 5** | x10 buffer | 2.5 |
| **(multi 2)** | CorRu12-F (10 µmol) | 3 |
| | CorRu12-R (10 µmol) | 3 |
| | dNTP (2.5 mmol) | 2 |
| | taq (5 U/µL) | 0.15 |
| | $ddH_2O$ (µL) | 13.35 |
| | Total (µL) | 24 |

Appendix 4: Five PCR settings used in this study, corresponding to master mixes 1-5 in Appendix 3. Repeats refer to number of times to repeat steps 2-4. Forever refers to a setting that remains until the samples are removed from the machine. The volume in each tube is 25 µL (24 µL Master mix, 1 µL DNA extract). Note that PCR-4 and PCR-5 are identical, since CorRu4 and CorRu5 were unsuccessful when multiplexed.

**PCR-1 (Crup6, CorRu11, CorRu33)**

| Step | 1 | 2 | 3 | 4 | Repeats | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Temperature (°C) | 94 | 94 | 58 | 72 | | 60 | 12 |
| Time (seconds) | 180 | 30 | 30 | 60 | | 1800 | Forever |
| | | | | | ←24 | | |

**PCR-2 (CorRu2, CorRu3)**

| Step | 1 | 2 | 3 | 4 | Repeats | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Temperature (°C) | 94 | 94 | 63 | 72 | | 60 | 12 |
| Time (seconds) | 180 | 30 | 30 | 60 | | 1800 | Forever |
| | | | | | ←26 | | |

**PCR-3 (Crup1)**

| Step | 1 | 2 | 3 | 4 | Repeats | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Temperature (°C) | 94 | 94 | 52 | 72 | | 60 | 12 |
| Time (seconds) | 180 | 30 | 30 | 60 | | 1800 | Forever |
| | | | | | ←26 | | |

**PCR-4 (CorRu4)**

| Step | 1 | 2 | 3 | 4 | Repeats | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Temperature (°C) | 94 | 94 | 57 | 72 | | 60 | 12 |
| Time (seconds) | 180 | 30 | 30 | 60 | | 1800 | Forever |
| | | | | | ←26 | | |

**PCR-5 (CorRu12)**

| Step | 1 | 2 | 3 | 4 | Repeats | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Temperature (°C) | 94 | 94 | 57 | 72 | | 60 | 12 |
| Time (seconds) | 180 | 30 | 30 | 60 | | 1800 | Forever |
| | | | | | ←26 | | |

Appendix 5: Scoring success rate of 8 microsatellite loci for 480 samples across all sites.

| Locus | Crup6 | CorRu11 | CorRu33 | CorRu2 | CorRu3 | Crup1 | CorRu4 | CorRu12 |
|---|---|---|---|---|---|---|---|---|
| Scoring success (%) | 95 | 90 | 92 | 81 | 80 | 86 | 88 | 86 |

Appendix 6: Results of Hardy-Weinberg exact test in GenePop, showing P-values and the inbreeding coefficient ($F_{IS}$) for all 64 locus-population combinations, ordered by P-value. P-values after applying the Benjamini-Hochberg false discovery rate (FDR) method are shown, with significant deviations from Hardy-Weinberg equilibrium in bold.

| Locus | Population | P-value | $F_{IS}$ | FDR P-value |
|---|---|---|---|---|
| CorRu11 | Kor | 0.0000 | 0.246 | **0.0000** |
| CorRu11 | SK01 | 0.0000 | 0.234 | **0.0000** |
| CorRu11 | TL | 0.0000 | 0.568 | **0.0000** |
| CorRu2 | TL | 0.0000 | 0.274 | **0.0000** |
| CorRu3 | Kor | 0.0000 | 0.415 | **0.0000** |
| CorRu3 | Lus-Out | 0.0000 | 0.518 | **0.0000** |
| CorRu3 | Mas | 0.0000 | 0.390 | **0.0000** |
| CorRu3 | SK08 | 0.0000 | 0.338 | **0.0000** |
| CorRu3 | TL | 0.0000 | 0.588 | **0.0000** |
| Crup1 | SK01 | 0.0000 | 0.876 | **0.0000** |
| CorRu11 | Lus-Out | 0.0001 | 0.250 | **0.0006** |
| CorRu11 | Lus-In | 0.0004 | 0.289 | **0.0021** |
| Crup1 | Kor | 0.0005 | 0.333 | **0.0025** |
| Crup1 | Mas | 0.0011 | 0.422 | **0.0050** |
| CorRu2 | SK01 | 0.0012 | 0.314 | **0.0051** |
| Crup1 | TL | 0.0015 | 0.138 | **0.0060** |
| CorRu3 | SK16 | 0.0019 | 0.598 | **0.0072** |
| Crup1 | Lus-Out | 0.0049 | 0.624 | **0.0174** |
| CorRu2 | Lus-In | 0.0100 | 0.306 | **0.0337** |
| Crup1 | SK08 | 0.0172 | 0.477 | 0.0550 |
| CorRu4 | TL | 0.0277 | 0.048 | 0.0844 |
| CorRu11 | Mas | 0.0414 | 0.173 | 0.1204 |
| CorRu2 | Mas | 0.0422 | 0.171 | 0.1174 |
| CorRu12 | Lus-Out | 0.0469 | 0.198 | 0.1251 |
| CorRu3 | Lus-In | 0.0573 | 0.305 | 0.1467 |
| Crup6 | Mas | 0.0686 | 0.106 | 0.1689 |
| CorRu2 | SK16 | 0.0703 | 0.196 | 0.1666 |
| CorRu33 | TL | 0.0705 | 0.147 | 0.1611 |
| CorRu11 | SK16 | 0.0944 | 0.082 | 0.2083 |
| Crup6 | Kor | 0.0968 | 0.024 | 0.2065 |
| Crup6 | SK16 | 0.1248 | 0.123 | 0.2577 |
| Crup1 | Lus-In | 0.1318 | 0.479 | 0.2636 |
| CorRu4 | Lus-In | 0.1602 | 0.078 | 0.3107 |
| CorRu12 | SK01 | 0.1880 | -0.002 | 0.3539 |
| CorRu4 | SK01 | 0.2370 | 0.139 | 0.4334 |
| CorRu2 | SK08 | 0.2600 | 0.170 | 0.4622 |
| CorRu11 | SK08 | 0.2635 | 0.218 | 0.4558 |
| CorRu33 | SK01 | 0.2866 | 0.021 | 0.4827 |
| Crup6 | Lus-Out | 0.3293 | 0.037 | 0.5404 |
| Crup6 | Lus-In | 0.3479 | 0.082 | 0.5566 |
| CorRu2 | Lus-Out | 0.3557 | 0.109 | 0.5552 |
| CorRu4 | Mas | 0.3989 | -0.144 | 0.6078 |
| CorRu12 | Kor | 0.4126 | -0.083 | 0.6141 |
| Crup1 | SK16 | 0.4344 | 0.236 | 0.6319 |
| CorRu33 | Lus-Out | 0.4471 | 0.111 | 0.6359 |
| CorRu12 | TL | 0.5814 | -0.050 | 0.8089 |
| Crup6 | SK01 | 0.5859 | -0.031 | 0.7978 |

| | | | | |
|---|---|---|---|---|
| CorRu12 | Mas | 0.5869 | 0.034 | 0.7825 |
| CorRu3 | SK01 | 0.5878 | 0.159 | 0.7677 |
| Crup6 | SK08 | 0.5966 | -0.011 | 0.7636 |
| CorRu4 | Kor | 0.5994 | 0.035 | 0.7522 |
| CorRu33 | SK16 | 0.6103 | -0.326 | 0.7511 |
| CorRu4 | Lus-Out | 0.6695 | 0.192 | 0.8085 |
| CorRu12 | SK08 | 0.6707 | -0.037 | 0.7949 |
| CorRu33 | Lus-In | 0.7026 | -0.025 | 0.8176 |
| CorRu33 | Kor | 0.7070 | -0.027 | 0.8080 |
| CorRu4 | SK08 | 0.7196 | -0.116 | 0.8080 |
| CorRu12 | Lus-In | 0.7404 | -0.028 | 0.8170 |
| CorRu4 | SK16 | 0.7733 | -0.167 | 0.8388 |
| CorRu12 | SK16 | 0.8116 | -0.057 | 0.8657 |
| Crup6 | TL | 0.9198 | -0.077 | 0.9650 |
| CorRu33 | Mas | 0.9288 | 0.008 | 0.9588 |
| CorRu2 | Kor | 0.9950 | 0.026 | 0.9950 |
| CorRu33 | SK08 | 0.9885 | -0.129 | 1.0042 |

Appendix 7: Pairwise genic differentiation output from GenePop, showing Chi$^2$, degrees of freedom (df) and P-values. P-values displayed as "Highly sign." in GenePop are shown as <0.001. Significant values after Benjamini-Yekutieli FDR correction are in bold. Values that remain significant after removing the flagged loci (Crup1, CorRu2, CorRu3, CorRu11) are shown by *.

| Population 1 | Population 2 | Chi$^2$ | df | P-value |
|---|---|---|---|---|
| Kor | Lus-In | Infinity | 16 | **<0.001** |
| Kor | Lus-Out | Infinity | 16 | **<0.001*** |
| Kor | Mas | Infinity | 16 | **<0.001*** |
| Kor | Sk01 | 22.447 | 16 | 0.129 |
| Kor | Sk08 | 22.071 | 16 | 0.141 |
| Kor | Sk16 | 18.716 | 16 | 0.284 |
| Kor | TL | Infinity | 16 | **<0.001*** |
| Lus-In | Lus-Out | Infinity | 16 | **<0.001*** |
| Lus-In | Mas | 70.192 | 16 | **<0.001*** |
| Lus-In | SK01 | Infinity | 16 | **<0.001** |
| Lus-In | SK08 | Infinity | 16 | **<0.001*** |
| Lus-In | SK16 | 43.970 | 16 | **<0.001** |
| Lus-In | TL | Infinity | 16 | **<0.001*** |
| Lus-Out | Mas | Infinity | 16 | **<0.001*** |
| Lus-Out | SK01 | Infinity | 16 | **<0.001** |
| Lus-Out | SK08 | Infinity | 16 | **<0.001** |
| Lus-Out | SK16 | 47.341 | 16 | **<0.001** |
| Lus-Out | TL | Infinity | 16 | **<0.001*** |
| Mas | SK01 | Infinity | 16 | **<0.001*** |
| Mas | SK08 | Infinity | 16 | **<0.001*** |
| Mas | SK16 | Infinity | 16 | **<0.001** |
| Mas | TL | Infinity | 16 | **<0.001*** |
| SK01 | SK08 | 19.798 | 16 | 0.229 |
| SK01 | SK16 | 14.786 | 16 | 0.540 |
| SK01 | TL | Infinity | 16 | **<0.001*** |
| SK08 | SK16 | 14.794 | 16 | 0.540 |
| SK08 | TL | Infinity | 16 | **<0.001*** |
| SK16 | TL | Infinity | 16 | **<0.001*** |

Appendix 8: Pairwise genic differentiation output from GenePop after pooling Skagerrak temporal samples (termed SK), showing $Chi^2$, degrees of freedom (df) and P-values. P-values displayed as "Highly sign." in GenePop are shown as <0.001. Significant values after Benjamini-Yekutieli FDR correction are in bold. These values remained significant after removing the flagged loci (Crup1, CorRu2, CorRu3, CorRu11).

| Population 1 | Population 2 | $Chi^2$ | df | P-value |
|---|---|---|---|---|
| Kor | Lus-In | Infinity | 16 | **<0.001** |
| Kor | Lus-Out | Infinity | 16 | **<0.001** |
| Kor | Mas | Infinity | 16 | **<0.001** |
| Kor | SK | 28.341 | 16 | 0.029 |
| Kor | TL | Infinity | 16 | **<0.001** |
| Lus-In | Lus-Out | 67.375 | 16 | **<0.001** |
| Lus-In | Mas | 71.498 | 16 | **<0.001** |
| Lus-In | SK | Infinity | 16 | **<0.001** |
| Lus-In | TL | Infinity | 16 | **<0.001** |
| Lus-Out | Mas | Infinity | 16 | **<0.001** |
| Lus-Out | SK | Infinity | 16 | **<0.001** |
| Lus-Out | TL | Infinity | 16 | **<0.001** |
| Mas | SK | Infinity | 16 | **<0.001** |
| Mas | TL | Infinity | 16 | **<0.001** |
| SK | TL | Infinity | 16 | **<0.001** |

Appendix 9: Script for statistical tests performed in R (version 3.0.3), showing Analyses of Covariance (ANCOVAs) on length-weight and length-gutted weight, and Generalized Linear Models (GLMs) for the Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI).

```
## 1. ANCOVA (multiple linear model) on log PAFL and log weight.
# Import data
        skolest <- read.delim(pipe('pbpaste'))
# Start with full model
        mod1 <- lm(log.weight~log.pafl*locality, data=skolest)
        anova(mod1)
# Interaction is significant, so we stick with this model.
        summary(mod1)
        plot(mod1)
# relevel to view pairwise site comparisons.
        skolest$relevel.locality <- relevel(skolest$locality,ref="mas")
        mod1b <- lm(log.weight~log.pafl*relevel.locality, data=skolest)
        summary(mod1b)
        skolest$relevel.locality<-relevel(skolest$locality,ref="sk01")
        mod1c <- lm(log.weight~log.pafl*relevel.locality, data=skolest)
        summary(mod1c)
        skolest$relevel.locality<-relevel(skolest$locality,ref="sk08")
        mod1d <- lm(log.weight~log.pafl*relevel.locality, data=skolest)
        summary(mod1d)
        skolest$relevel.locality<-relevel(skolest$locality,ref="sk16")
        mod1e <- lm(log.weight~log.pafl*relevel.locality, data=skolest)
        summary(mod1e)
```

```
        skolest$relevel.locality<-relevel(skolest$locality,ref="tl")
        mod1f <- lm(log.weight~log.pafl*relevel.locality, data=skolest)
        summary(mod1f)
        skolest$relevel.locality<-relevel(skolest$locality,ref="lus.in")
        mod1g <- lm(log.weight~log.pafl*relevel.locality, data=skolest)
        summary(mod1g)


# Perform ANCOVA without Trondheimsleia samples
        skolest2 <- subset(skolest, locality !="tl")
        mod2 <- lm(log.weight~log.pafl*locality, data=skolest2)
        anova(mod2)
        summary(mod2)
        plot(mod2)
# relevel to view pairwise site comparisons.
        skolest2$relevel.locality<-relevel(skolest2$locality,ref="lus.in")
        mod2b <- lm(log.weight~log.pafl*relevel.locality, data=skolest2)
        summary(mod2b)
        skolest2$relevel.locality<-relevel(skolest2$locality,ref="lus.out")
        mod2c <- lm(log.weight~log.pafl*relevel.locality, data=skolest2)
        summary(mod2c)
        skolest2$relevel.locality<-relevel(skolest2$locality,ref="mas")
        mod2d <- lm(log.weight~log.pafl*relevel.locality, data=skolest2)
        summary(mod2d)
        skolest2$relevel.locality<-relevel(skolest2$locality,ref="sk01")
        mod2e <- lm(log.weight~log.pafl*relevel.locality, data=skolest2)
        summary(mod2e)
        skolest2$relevel.locality<-relevel(skolest2$locality,ref="sk08")
        mod2f <- lm(log.weight~log.pafl*relevel.locality, data=skolest2)
        summary(mod2f)


## 2. ANCOVA on log PAFL and log gutted weight.
# Import data
        gutted <- read.delim(pipe('pbpaste'))
# Start with full model
        mod3 <- lm(log.gutted~log.pafl*locality, data=gutted)
        anova(mod3)
        summary(mod3)
        plot(mod3)
# There is no significant interaction, so I perform another test without the
interaction term
        mod3b <- lm(log.gutted~log.pafl+locality, data=gutted)
        anova(mod3b)
        summary(mod3b)
        plot(mod3b)
# relevel to view pairwise site comparisons.
        gutted$relevel.locality<-relevel(gutted$locality,ref="lus.in")
        mod3c <- lm(log.gutted~log.pafl+relevel.locality, data=gutted)
        summary(mod3c)
        gutted$relevel.locality<-relevel(gutted$locality,ref="lus.out")
        mod3d <- lm(log.gutted~log.pafl+relevel.locality, data=gutted)
```

```
        summary(mod3d)

## 3. GLM on HSI and GSI between sites
# Import data
        condition <- read.delim(pipe('pbpaste'))
# Perform GLM on HIS
        fit.glm <- glm(hsi.proportion~locality, family='quasibinomial',
        data=condition)
        anova(fit.glm, test='F')
        summary(fit.glm)
# Tukey-HSD test
        library(multcomp)
        mc <- glht(fit.glm, linfct=mcp(locality='Tukey'))
        summary(mc)

# Perform GLM on GSI
        fit2.glm <- glm(gsi.proportion~locality, family='quasibinomial',
        data=condition)
        anova(fit2.glm, test='F')
        summary(fit2.glm)
# Tukey-HSD test
        mc2 <- glht(fit2.glm, linfct=mcp(locality='Tukey'))
        summary(mc2)
```